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# THE PATHOLOGY OF SECONDARY SHOCK*

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The term *secondary shock* is used here to imply a distinction from the uncomplicated effects of hemorrhage—*hemorrhagic shock*—and from *primary shock*. Hemorrhage is an obvious cause for low blood pressure and circulatory deficiency after injuries. Although the clinical signs are similar, the accompanying physiologic changes and the post-mortem findings after death from uncomplicated hemorrhage differ in several ways from those of secondary shock.[1]

Primary or "neurogenic shock" (Blalock [2]) is a neurovascular reaction like syncope or fainting. It may be excited by pain, emotional reactions, or perhaps by nerve impulses arising in damaged tissues (Phemister [3]). Primary shock develops promptly and usually is transient unless accompanied by extensive injury or hemorrhage; then it may merge into secondary shock with hemorrhage as a contributory factor. Combinations of neurogenic, hemorrhagic, and secondary shock in the same patient have caused confusion.[4] When the term shock is used without qualification in the succeeding pages, it will be understood to mean secondary shock, sometimes called collapse or peripheral circulatory failure.

## DYNAMICS OF SHOCK: RESUMÉ

Shock from wounds was a major problem during World War I. An investigation by eminent physiologists, pharmacologists, internists, and surgeons compared observations on wounded men with data from experimental studies. The results were summarized [5] as follows:

"The theory of secondary shock which has the strongest support, both in clinical observations and in laboratory experiments, is that of a toxic factor, arising from damaged and dying tissue and operating to cause an increased permeability of the capillary walls and a consequent reduction of blood volume by escape of plasma into the lymph spaces. Thus the concentration of the corpuscles is also readily explained. It is recognized that after a sufficient time infection may occur and be

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of such character in itself as to induce a persistent low blood pressure. According to this theory there might be no essential difference between the effects of toxins given off by damaged tissue and of toxins resulting from activity of bacteria."

Later investigations by many workers indicated the importance of hemorrhage and loss of blood and fluid in traumatized regions. No evidence of toxic substances in the blood was found by the methods then in use. Many believed that all of the phenomena of shock could be explained by local loss of blood and fluid.

The first published report on the pathology of shock [6] indicated that hyperemia and edema of viscera, petechial hemorrhages, serous effusions, and acute degeneration of parenchymatous tissues are pathologic changes characteristic of this condition. These changes in visceral areas, remote from the region of trauma, indicated the effects of factors other than loss of blood and fluid. Our subsequent investigations indicated that the syndrome of shock occurs also after burns, poisoning, severe infections, and intestinal obstruction. The same pattern of visceral changes was seen in men and animals after death from these conditions.

The dynamics of shock was interpreted in accordance with the principles of capillary physiology (Krogh,[7] Landis,[8] Lewis [9]). Various agents and conditions injurious to endothelium produce atony and dilatation of capillaries and venules. This increases the volume capacity of the vascular bed. Endothelium, when affected by such agents, including anoxia, becomes abnormally pervious to colloids. This allows plasma to escape into the tissue spaces, causing edema and a tendency to hemoconcentration. The loss of plasma lowers the *total* blood volume, and the stagnation of blood in dilated vessels lowers the *effective* blood volume. This leads to a disparity between the volume of blood and volume capacity of the vascular bed, and diminishes the return flow of venous blood to the heart. These effects produce a circulatory deficiency characterized by decreased blood volume and volume-flow of blood, reducing the amount of oxygen delivered to the tissues. Tissue anoxia *per se* causes capillary permeability [8] and thus introduces a self-perpetuating factor which causes the circulatory deficiency to increase progressively. This vicious circle, unless interrupted, leads to an irreversible stage and to death. It appears that capillary permeability and anoxia have reciprocal effects; either of them presently brings the other into action. Abnormal permeability of endothelium deranges fluid balance. A tendency to visceral edema, hemoconcentration, and to stasis results.

Subsequent investigations indicated the presence of some substance, absorbed from areas of tissue injury, which produces vasodepressor

effects. Best and Solandt,[10] by an exchange transfusion of blood between traumatized and normal dogs, produced circulatory deficiency in the untraumatized animals. Kendrick, Essex, and Helmholz [11] reported similar results from transfusion by a different technic. Freeman, Cullen, and Schecter [12] produced shock by trauma to limbs which had been taped to prevent excessive loss of blood and fluid locally. They found evidence of a toxic factor absorbed from the traumatized region. Similar results were obtained by others when shock was induced by tourniquet and by freezing.

Blalock [13] produced massive pressure on the muscles of the limbs, simulating the "crush syndrome." Lymph from the thoracic duct, injected into other dogs, caused hemoconcentration and a decline in blood pressure. He believed toxic substances, arising in the injured extremity, had entered the lymph vessels. Experimental crush injury [14] caused hemoconcentration and a fall in blood pressure, accompanied, in some instances, by hematuria and retention of nitrogenous wastes in the blood. Katzenstein, Mylon, and Winternitz [15] found that lymph from the thoracic duct during tourniquet shock caused a protracted fall in blood pressure, often ending fatally, when injected intravenously into other dogs.

Aub and associates [16] showed that bacterial contamination of injured muscles produced toxic substances in the lymph collected from those muscles. Prinzmetal, Freed, and Kruger [17] induced shock by excising and grinding muscle tissue aseptically, then implanting this pulp into the bed from which the muscle was excised. This caused death usually within 24 hours. They believed that shock-producing substances, resulting largely from bacterial contamination, were absorbed from the crushed muscle. It is recalled that all open traumatic wounds are grossly contaminated, and that bacterial growth proceeds rapidly in crushed devitalized tissues.

Summarizing the problems of shock, Blalock [18] found general agreement that traumatic shock is due to regional loss of blood, toxemia, or nerve impulses, separately or in combination. He stated that the search for and identification of the toxic factor or factors is the problem of first importance. Among other problems in the pathogenesis of shock, he listed that of determining the rôle of infection superimposed upon injury.

Shorr, Zweifach, and Furchgott [19] made tests for vasodepressor and vaso-excitor substances in blood and fluids from injured and from anoxic tissues. Their results led to the conclusion that the development of shock is due to vascular atony resulting from a preponderance of vaso-

depressor effects, and that anoxia is a factor causing the circulatory deficiency to progress in a "morbid cycle" to an irreversible stage. Heilbrunn and co-workers [20] demonstrated that water extracts of muscle and of tissues from heat-killed animals contained some dialyzable toxic substances which caused death when injected into other animals. Before death these animals showed symptoms like those of heat-treated (scalded or burned) animals.

Recent contributions, as noted above, support the explanation of *traumatic toxemia,* but with important modifications and additions:

(1) It has been demonstrated that, in traumatic or surgical shock, local loss of blood and fluid plays a rôle not recognized formerly. The importance of this factor is in direct proportion to the volume of blood and/or fluid lost. In some instances this is so great as to make it the dominant factor. In other cases it may be minor.

(2) Anoxia is highly important in the vicious circle by which shock tends to progress to an irreversible stage. The anoxia may be *anoxic* as in asphyxia, or *anemic* as when the red corpuscles have been reduced below physiologic limits by hemorrhage or otherwise, or *stagnant* as by circulatory stasis, or *toxic* as from the effects of poisons upon the tissue cells (Blalock [2]). In any instance, anoxia tends to cause atony and permeability of capillary walls resulting in progressive circulatory deficiency and death. Anoxia is the factor which ultimately "stops the machine and wrecks the machinery."

(3) Comprehension of the mechanism of shock has led to the recognition of the same syndrome in conditions other than trauma, extensive surgery, and burns. Atchley [21] cited instances in patients with severe infection, diabetic acidosis, Addison's disease, bile peritonitis, vaccine reactions, heat prostration, and snake bites. These conditions led to stasis of blood in the capillaries, generalized anoxemia, capillary dilatation, and leakage of plasma into the tissues, operating in a vicious circle. Harkins [22] stated that shock may occur in association with a wide variety of conditions: hemorrhage; mechanical or operative trauma; burning; freezing; heat stroke; radiation burns; sunburn; asphyxia; vascular occlusion; intestinal strangulation; application of tourniquet; bile peritonitis; perforated gastric ulcer; pancreatitis; various poisons such as $HgCl_2$, arsenic, gold chloride, snake venoms; special capillary poisons such as products of tissue autolysis, histamine, peptone; medical conditions such as an anaphylaxis, diabetic coma, eclampsia; severe infections such as cholera, streptococcal and influenzal pneumonia, gas gangrene, diphtheria, peritonitis; and various anesthetic agents.

A condensed summary on the mechanism and associated features was issued by the Subcommittee on Shock [23] of the National Research Council. It set forth that the outstanding physiologic feature is peripheral circulatory failure caused by a discrepancy between the capacity of the vascular system and the volume of fluid which it contains. Shock was defined as the *clinical condition characterized by progressive reduction in circulating blood volume due to increased capillary permeability*. Any agency which affects the permeability of the vessels allowing the escape of plasma proteins, may lead to shock. This is accompanied by a progressive reduction of circulating blood volume due to the escape of plasma. Anoxia is a factor of prime importance causing capillary permeability and impaired circulation. This summary closed with a brief statement on associated tissue changes:

"The pathologic picture which is found when the tissues are examined after death from shock is that to be expected from peripheral circulatory failure. There is widespread congestion and engorgement of the capillaries and venules throughout the body . . . . This congestion is found throughout the viscera and in the lungs. There is edema in the tissue spaces and effusion in the serous cavities . . . . The impairment of circulation affects other organs as well. Necrosis of liver cells, liquefaction of the suprarenal medulla, and congestion of the pancreas are observed. The kidneys show evidence of parenchymatous degeneration. Patches of capillary hemorrhage occur in the medulla, and numerous red cells are found within the tubules in such regions. The pathologic picture thus confirms the clinical and experimental evidence on the significance of increased capillary permeability as the essential feature of shock."

The preceding data indicate that divergent views have been adjusted concerning the major factors in the dynamics of shock. Investigations on other features can now go forward relatively undisturbed by controversial discussions.

## Material and Methods

The purpose of this survey is to secure information on the occurrence of shock from causes other than trauma, to collect additional data on associated pathologic changes, and to record evidence of physiologic disturbances, especially the renal effects.

The Director of the Army Institute of Pathology provided opportunity to study the material collected there. The clinical and postmortem records, histologic preparations, and the entire facilities of the Institute were made available for a survey on the pathology of shock as seen in the personnel of the U. S. Army. Thousands of records of death from all causes presented an abundance of material for study. To tabulate the data from all of these was an undertaking of staggering proportions, for which time was not available. Accordingly, the statistical approach was abandoned in favor of detailed studies of representative cases appropriately grouped. In every instance the

clinical and post-mortem data were taken directly from the records, but the microscopic studies were made by me.

## Trauma

Under the heading of trauma are grouped those instances in which fatal circulatory deficiency resulted from traumatic or surgical injuries. The causative mechanism in such cases includes trauma, hemorrhage, infection (all battle wounds are grossly contaminated), and sometimes anesthesia, surgery, reaction to transfusions, renal complications and other contributory conditions. It is manifestly impossible to evaluate the relative weight of these several factors in any given case. The causation of shock in this group is more complex than in the succeeding groups.

Thirty cases of shock resulting from trauma and associated conditions were studied. They presented different degrees of severity as indicated by the interval between injury and death, ranging from a few hours to 6 days. These features are indicated briefly in the list which follows:

| *A.I.P. Accession Number* | *Origin* |
|---|---|
| 84837 | Abdomen hit by the recoil of a 90 mm. gun. Death 8 hours later. |
| 122323 | Death during surgical operation for G.S.W.* received weeks before. |
| 122486 | Death during surgical operation for multiple G.S.W. |
| 128121 | S.F.W.* of thighs, compound fracture, transfusion reaction, anuria. |
| 126488 | G.S.W. of legs and feet, compound fracture. Death 24 hours later. |
| 122293 | G.S.W. of thigh, fractured femur. Death 24 hours later. |
| 86750 | G.S.W. of body, fractures. Death 24 hours later. |
| 114945 | G.S.W., multiple fractures. Death 30 hours later. |
| 88192 | Automobile accident, multiple fractures, gas gangrene. Death 34 hours later. |
| 86134 | Fall from fourth story window, compound fractures. Death 36 hours later. |
| 97750 | Automobile accident, fractures, internal injuries. Death 48 hours later. |
| 118315 | S.F.W. of thighs, pulmonary edema. Death 48 hours later. |
| 118316 | G.S.W. of leg and thigh, fractures. Death 48 hours later. |
| 127136 | S.F.W. of legs and feet, compound fracture, anuria. Death 48 hours later. |
| 84641 | Automobile accident, internal injuries, anuria, high nonprotein nitrogen. Death 53 hours later. |
| 85677 | Automobile accident, fractures of femurs, mandibles, ribs. Death 3 days later. |
| 126438 | G.S.W. of arm, fractures, gas gangrene, amputation. Death 3 days later. |
| 127580 | S.F.W. of buttocks, pulmonary edema, anuria. Death 3 days later. |
| 102060 | Resection of rectal carcinoma, oliguria, uremia. Death 4 days later. |
| 119159 | Automobile accident, compound fracture of pelvis. Death 4 days later. |

* G.S.W. and S.F.W. indicate gunshot wounds and shell fragment wounds, respectively.

122144 S.F.W., comminuted fractures of legs and arms, gas gangrene, oliguria. Death 4 days later.
113425 Ileitis, surgical resection, anuria, high nonprotein nitrogen. Death 5 days later.
118404 S.F.W. of thighs and leg, compound fractures, anuria, high nonprotein nitrogen. Death 5 days later.
121439 G.S.W., transfusion reaction, anuria. Death 5 days later.
118313 G.S.W. of legs, transfusion (reaction?), high nonprotein nitrogen. Death 6 days later.
121411 S.F.W. of legs, comminuted fractures, shock developed 6 days later.
128117 S.F.W., multiple fractures, anuria, uremia. Death 6 days later.
126031 S.F.W., gas gangrene, amputation. Death 6 days after receipt of wound.
127269 G.S.W. of leg, ischemic gangrene. Death 6 days later.
118359 S.F.W. of buttocks and legs, fractures, gas gangrene, amputation. Death 10 days after receipt of wound.

### *Pathologic Conditions*

Pulmonary hyperemia and edema were found regularly in all cases. In most instances these were marked; they were slight or moderate in only 6. Serous effusions were present in 21. Petechiae were noted at necropsy or found microscopically in 15. The development of pneumonia in shock with delayed death has been reported.[24] In 20 cases death occurred 48 hours or longer after the injury; terminal pneumonia was present in 12 of these, either incipient or well advanced (Figs. 1 and 10).

Some degree of atelectasis was present in 13 cases. In some this was noted grossly at necropsy; in others it was seen in varying degrees microscopically, as small scattered areas. This feature has not been reported hitherto as associated with shock. Its significance and mechanism of origin are not apparent.

Parenchymatous degeneration ranging to necrosis was a regular feature, especially in the kidneys, liver, and heart. In 20 cases, marked parenchymatous degeneration was present in the kidneys; in 12 of these, necrosis of tubular epithelium also was present; in 6 cases the degeneration was moderate or slight. In the remaining 2 instances, post-mortem changes made the recognition of degeneration uncertain. Casts were present in the tubular lumina in 19 instances; these were hyaline, granular, cellular, or mixed; in a few instances the casts contained brown pigment. In all instances the renal parenchyma was moderately or markedly hyperemic; minute hemorrhages were seen frequently, and red cells were often found in the tubular lumina (Figs. 4 and 5).

In 26 cases, parenchymatous degeneration of hepatic cells was marked; in 22 there was also necrosis. This varied from occasional scattered cells, focal necrosis, to extensive areas usually in the central

part of the lobules (Fig. 3). Degenerative changes of heart muscle fibers, ranging from slight to marked, were present in 21 instances. In a few cases foci of necrosis were present in lymphoid tissue and in the adrenals (Fig. 2). Splenic engorgement was noted in 13 instances.

In these cases, shock resulted not from a single causative agent but from a combination of causes: the delayed effects of tissue injury, hemorrhage, infection, and, in some instances, anesthesia, surgery, and unfavorable reaction to transfusion. The relative importance of these factors varied from case to case.

Hemorrhage and local loss of blood had occurred in many cases. That hemorrhage was not the sole factor was suggested by the facts: (1) that restoration of blood volume by fluids, plasma, and transfusion did not restore circulatory efficiency; (2) that in some instances a high red blood cell count indicated that some mechanism had disturbed fluid balance and had prevented the dilution of blood which normally follows hemorrhage; (3) that marked visceral congestion, edema, effusions, and petechiae indicated the effects of some other agency. These findings are not characteristic of death from uncomplicated hemorrhage.

In some instances infection was not apparent from the clinical or post-mortem data, while in battle wounds, some complicated by gas gangrene, infection was a prominent contributory factor. The visceral findings in these were like those seen throughout the group. There is evidence that products of bacterial growth may cause capillary permeability. Examinations of the blood, when recorded, showed varying degrees of hemodilution; in only 3 instances was there hemoconcentration. However, most cases had received fluid intravenously which would, of course, counteract hemoconcentration. In some, the large amounts of fluid given were more, perhaps, than a failing circulation could retain. Some degree of capillary permeability is a central feature in shock; such capillaries will allow saline fluids to escape rapidly, thus increasing the edema.

The circulatory disturbances which lead to shock may develop in varying degrees and with varying rapidity. Maximal degrees may cause death from failure of the circulation within 24 hours regardless of therapeutic measures. With lesser degrees the patient may respond favorably and recover; in others, a condition of sublethal shock persists for several days. In such cases, either renal insufficiency or terminal pneumonia may be expected to complicate the condition.

Oliguria or anuria is present early and continues as shock progresses (Atchley,[25] Freeman,[23] Blalock[2]). This often leads to uremia if the subject survives for a few days.[26] Renal deficiency and retention of nitrogenous wastes were present in 8 cases of this group and 3 of these followed transfusion reactions. A cause for renal insufficiency is suggested in the marked degeneration and necrosis of the tubular epithelium.

Transfusion reactions vary widely in degree. The severest reactions cause death from progressive circulatory collapse, sometimes resembling fatal anaphylactic shock (Best and Taylor,[27] Bordley,[28] Wiener[29]). Renal deficiency develops following severe nonfatal reactions and death may occur from uremia, as in cases 121439, 127136, and 128121. In these the visceral findings were the same as in delayed death after shock from other causes. Degenerative changes and necrosis of renal tubular epithelium were marked, as in other instances of uremia associated with shock.

Traumatic shock results from a combination of causes, the relative importance of which varies in different cases. The causative factors in subsequent groups do not include so many variables, hence an analysis of the mechanism will be less involved. Early death from traumatic shock results from circulatory failure. Death after 48 hours often is the result of a combination of circulatory deficiency with renal deficiency and terminal pneumonia.

## Burns

Patients with extensive burns of the skin often present the complete picture of secondary shock. Harkins'[30] survey indicated that shock is the cause of death in 60 to 75 per cent of fatalities from burns. Examination of data from numerous cases indicated that they confirm what is already recorded in medical literature concerning the clinical course and the post-mortem findings after death from burns. Ten representative cases were studied for comparison with the findings in shock from other causes. Also 3 instances of burns made by phosphorus upon the skin or blown into the flesh were studied. In these, absorption introduced a possible factor of phosphorus poisoning.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 87628 | Extensive 2nd and 3rd degree burns. Death 12 hours later. |
| 122578 | Extensive 2nd and 3rd degree burns, about 40 per cent of skin. Death 12 hours later. |
| 125364 | Extensive 2nd and 3rd degree burns. Death 15 hours later. |

| | |
|---|---|
| 125903 | Extensive 2nd and 3rd degree burns. Death 24 hours later. |
| 107676 | Extensive 3rd degree burns, high nonprotein nitrogen. Death 28 hours later. |
| 86891 | Fractures, 2nd and 3rd degree burns. Death 26 hours later. |
| 127272 | Extensive 2nd and 3rd degree burns. Death 48 hours later. |
| 122679 | Burns of entire body, anuria. Death 3 days later. |
| 90393 | Extensive severe burns, anuria. Death 4 days later. |
| 118506 | Extensive 2nd and 3rd degree burns, high nonprotein nitrogen. Death 8 days later. |
| 114072 | Phosphorus burns, 40 per cent of skin. Death 12 hours later. |
| 93453 | Phosphorus burns, 2nd and 3rd degree. Death 15 hours later. |
| 106660 | Phosphorus burns, 2nd and 3rd degree, anuria, high nonprotein nitrogen. Death 4 days later. |

In every case the findings were like those of traumatic shock, but tended to be more severe. Pulmonary hyperemia and edema were marked (Fig. 6). Pleural effusions were noted in 7, and petechiae in 9 instances. Terminal pneumonia was present in the lungs of 3 men who lived longer than 2 days after burning. The degeneration and necrosis of renal tubular epithelium were marked; degeneration and necrosis of hepatic cells were extensive (Fig. 9). In 10 instances the adrenal cortices were degenerated and abnormally vacuolated. In a few instances, post-mortem changes made the recognition of degeneration uncertain. Myocardial degeneration was seen in 12 of the 13 cases. Casts were found in renal tubules in 7 instances. In one of these the casts contained quantities of brown pigment; no transfusion or sulfonamide therapy had been given in this case (Figs. 8 and 9).

Some writers disregard the systemic effects of burns and attribute depleted blood volume and hemoconcentration to local loss of fluid in and about the burned areas. The importance of such loss is unquestionable; its effects are proportional to the amount of fluid lost. But loss of fluid locally does not account for the intense hyperemia, edema, acute degeneration and necrosis occurring in visceral areas remote from the burn. These indicate widespread and serious *systemic* effects. No treatise on burns will be complete unless it provides an acceptable explanation for the systemic as well as the local effects.

It has been suggested that degeneration and necrosis may result from the absorption of tannic acid, sulfonamides, or other drugs applied to the burns. It should be remembered that visceral hyperemia, edema, and degeneration and necrosis of hepatic and renal cells were set forth as characteristic features long before such local treatments were practiced (Bardeen,[31] Pack [32]). Significant evidence has accumulated indicating the systemic effects of toxic products absorbed from the burned tissue itself, and no evidence incompatible with this interpretation has been shown.

## Poisoning

It has been observed that acute poisoning often is accompanied by signs of shock. These include weakness, perspiration, vomiting, rapid pulse, low blood pressure, and oliguria. Hemoconcentration usually accompanies this syndrome.[33] Often the visceral changes, including hyperemia, parenchymatous degeneration, edema, and petechial hemorrhages, are like those already described. These effects result from various poisons, hence they are not the specific effect of some particular drug or chemical. It is of interest to compare the changes seen in the viscera after death by poisoning with those seen after shock from trauma and from burns. Data and post-mortem evidence from 20 cases of poisoning were studied.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 89731 | Shock reaction, delayed 3 days after neoarsphenamine. Death 24 hours later. |
| 111925 | Immediate reaction to mapharsen, oliguria, high nonprotein nitrogen, hemoconcentration, uremia. Death 7 days later. |
| 120574 | Arsenic poisoning, oliguria, high nonprotein nitrogen, hemoconcentration. Death 36 hours later. |
| 128449 | Suicide by arsenic, shock. Death same day. |
| 129165 | Suicide by arsenic, shock, hemoconcentration. Death 28 hours later. |
| 60482 | Mercuric poisoning, anuria, hemoconcentration. Death 2 days later. |
| 66984 | Suicidal mercuric poisoning, anuria, high nonprotein nitrogen. Death 8 days later. |
| 98365 | Mercuric poisoning, shock, hemoconcentration. Death 24 hours later. |
| 72500 | Suicide, rat poison (phosphorus). Death 7 days later. |
| 85342 | Suicide, rat poison (phosphorus). Death 9 hours later. |
| 98461 | Suicide, rat poison (phosphorus), shock. Death 5 days later. |
| 103527 | Overdose of barbiturate. Death 24 hours later. |
| 106299 | Found dead, barbiturate poisoning. |
| 106544 | Seconal poisoning, shock, found unconscious. |
| 108088 | Found dead, barbiturate poisoning. |
| 116989 | Suicide by barbiturate. Death 2 days later. |
| 113935 | Alcoholism and barbiturate poisoning. Death 48 hours later. |
| 128452 | Suicide by barbiturate poisoning, shown by chemical analysis. |
| 128656 | Barbiturate poisoning, shown by chemical analysis. |
| 128611 | Protracted drinking bout, acute alcoholism. |

Pulmonary hyperemia and edema were present in marked degree in 13; in 6 they were slight or moderate; and in one, suicide by arsenic, they were absent. Atelectasis was seen in 4 instances. Terminal pneumonia was found in 4 of the 7 men who lived 48 hours or longer. Petechial hemorrhages were recorded in 12, serous effusions in only 4. Degeneration and necrosis of renal tubular epithelium were found regularly, regardless of the type of poisoning. Necrosis was more marked after poisoning with arsenicals, mercury, and phosphorus than

after barbiturate poisoning; also, fewer casts were found in the tubules in the latter. Likewise degeneration and necrosis of hepatic cells were regularly present, but were less marked after barbiturate poisoning. Myocardial degeneration was seen in 13 cases; in 7 cases no histologic section of heart muscle was saved. Splenic engorgement was present in 13 cases.

Traumatic shock results from the combined effects of several causative and contributory conditions, hence the difficulty of accurate analysis, but shock from the effect of poisons is less complicated since the contributory factors are few. There is no possibility of nerve impulses from wounded tissues, of hemorrhage or leakage of plasma locally, of infection, anesthesia, or reaction to transfusion of blood or plasma. These indeterminate variables have been eliminated from the equation, making analysis relatively simple.

Students of capillary reactions [7, 9, 34] have shown that various drugs and chemicals, sometimes called "capillary poisons," may cause atony, dilatation, and increased permeability of capillary endothelium. Any kind of injury to capillaries increases the permeability of their walls.[9] These considerations indicate that various poisons may produce shock by their direct effects upon capillary endothelium. This interpretation is supported by the visceral findings; the capillovenous hyperemia, edema, serous effusions, and petechiae indicate atony, dilatation, and abnormal permeability of the walls of the minute vessels. Such changes did not vary in kind or degree with the various types of poison. The renal effects of poisons, especially mercurials and arsenicals, are well known. These are considered in a later section.

### Infections

Internists have observed that severe fulminating infection may cause progressive circulatory failure like that of shock from other causes. This effect does not depend upon the bacterial species, but upon the virulence in the individual case. Ten cases of severe infection, as listed below, were studied.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 118865 | Fulminating meningitis. Death 12 hours later. |
| 93449 | Fulminating meningitis. Death 12 hours later. |
| 106479 | Fulminating meningitis. Death 24 hours later. |
| 128538 | Fulminating meningitis. Death 24 hours later. |
| 108004 | Mixed streptococcal and staphylococcal meningitis. |
| 101617 | Septicemia (organism not stated). Death 6 hours later. |
| 108955 | Streptococcal septicemia. Death 7.5 hours later. |

| | |
|---|---|
| 102147 | Influenza. Death 48 hours later. |
| 111409 | Severe infection (tularemia?), anuria, high nonprotein nitrogen, "uremic frost." Death 12 days later. |
| 125907 | Falciparum malaria, anuria, high nonprotein nitrogen. Death 9 days later. |

The pathologic findings in each of these cases presented the same degeneration ranging to necrosis, as seen in shock from trauma, burns, and from poisons. Pulmonary hyperemia and edema were intense in 9 cases, moderate in one (93449). Petechiae were present in 9 and absent in one (111409). Hepatic, renal, and cardiac degeneration and necrosis were marked in all cases. Two of the 4 cases of fulminant meningitis—Waterhouse-Friderichsen syndrome—had extensive adrenal hemorrhages. In one case these were absent and in one no observations on the adrenals were recorded and sections of them were lacking.

Two patients, cases 125907 and 111409, died of uremia on the 9th and 12th days, respectively. In one, terminal pneumonia also was present. Each showed extensive degeneration and necrosis of renal tubular epithelium, casts, and débris in the tubular lumina. Similar renal findings were present in the patient with influenza dying in 48 hours.

The occurrence of circulatory collapse is not unusual in diphtheria, septicemia, meningitis, gas gangrene, cholera, plague, yellow fever, malaria, and in other acute infections. Its occurrence seems to depend on the severity or virulence of the infection. Dale [35] stated that the action of bacterial ferments upon proteins may give rise to cleavage products which will cause secondary shock. Atchley [21] believed that this results from paralysis of the capillaries by bacterial toxins. He cited an instance of pneumococcal vaccine given intravenously, resulting in shock. Brodie [36] found that small amounts of diphtheria toxin caused an immediate fall in blood pressure when given intravenously to animals. MacCallum [37] made similar observations. Harding [38] surveyed 800 cases of diphtheria and noted that circulatory failure, with hemoconcentration like that of wound shock, occurred in the toxemic stage. Influenza of fulminating severity produces progressive circulatory deficiency ending in death. Other instances might be cited.

Shock in severe infections, like that resulting from poisons, is relatively simple in its origin; trauma, hemorrhage, anesthesia, local loss of fluid and other complicating factors are not involved. Many believe that bacterial toxins, or products of bacterial metabolism in the tissues, affect endothelium like histamine or other capillary poisons. This

interpretation is supported by necropsy findings in such cases. These are not different in kind from those seen in shock from other causes.

It is recognized by internists that severe infections often cause deficient renal function. This occurrence is not limited to yellow fever and falciparum malaria but is seen with other infections. Two of the cases studied showed this feature. The gross and microscopic changes in the kidneys were like those accompanying renal failure in the other groups.

### Anoxia

The importance of *anoxia* in the mechanism of secondary shock has been emphasized by many authors. It is believed to be a major factor in the vicious circle by which shock progresses to an irreversible stage. For this reason, findings after death from simple lack of oxygen, or from asphyxia, are of interest. A group of 15 such cases was studied.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 83172 | Removed $O_2$ mask in low pressure chamber, equivalent altitude 36,500 ft. |
| 108338 | Removed $O_2$ mask in flight at 23,000 ft. |
| 111237 | $O_2$ line disconnected in flight at 27,000 ft. |
| 114331 | $O_2$ line disconnected in flight at 30,000 ft. |
| 114333 | $O_2$ line frozen in flight at 30,000 ft. |
| 114342 | $O_2$ line disconnected at 31,500 ft. |
| 114334 | $O_2$ line disconnected at 30,000 ft. |
| 114356 | $O_2$ tank empty at 26,000 ft. |
| 114357 | $O_2$ line frozen at 26,000 ft. |
| 100788 | Asphyxiated by CO. |
| 128137 | Asphyxiated by CO. |
| 128450 | Asphyxiated by CO. |
| 129055 | Asphyxiated by CO. |
| 127170 | Suffocation by fumes in burning building. |
| 128153 | Death by drowning. |

Ten additional records of death by anoxia in aviation were reviewed. In these the necropsies were done 24 to 48 hours after death, hence observations on parenchymatous changes were unsatisfactory. The circulatory changes in these 10 were exactly like those given below, but figures from these are not included in the findings.

The visceral changes were like those in the preceding groups except that hyperemia was more intense and necrosis and edema were less marked. In each case there was intense hyperemia of the lungs, liver, and kidneys. Petechial hemorrhages were noted in the post-mortem records in each instance. Edema of the lungs was marked in 3, moderate in 8, and absent in 4 cases; serous effusions were not recorded in any instance. Parenchymatous degeneration of the liver and kidneys was marked in 8 cases, slight in one; in 6 instances, post-mortem

changes made the recognition uncertain. Myocardial degeneration was seen in only 7 instances. Some degree of necrosis of both hepatic and renal tubular cells was seen in 8 cases.

The time interval between the beginning of anoxia and the cessation of circulation is not known; however, it probably was short, perhaps 15 to 30 minutes. Perhaps this was insufficient time for the development of edema and effusions. The presence of severe parenchymatous degeneration and of beginning necrosis deserves comment. Since they developed in so short a time, it appears that renal and hepatic epithelium is delicately susceptible to anoxia, even of short duration. It is significant also that petechial hemorrhages were seen in every case.

The cause of death in this group was simple. Neither nerve impulses from traumatized tissues, hemorrhages, local loss of fluid, poisonous substances, bacterial toxins, nor products of tissue autolysis were possible factors. Yet the visceral changes were of the same pattern as when one or more of the factors just mentioned were operative. Anoxia appears to be the one common factor in the mechanism of secondary shock from diverse causes. Perhaps it is the most important cause for petechial hemorrhages and for the parenchymatous changes found after death by shock.

When men succumbed to lack of oxygen in high altitude flight, the additional factor of low atmospheric pressure was introduced. The intense hyperemia, extreme dilatation of capillaries and venules, and the occurrence of numerous petechiae may be due in part to decreased extravascular (atmospheric) pressure. This would tend also to cause edema. Hyperemia, edema, and petechiae can be produced locally by applying a vacuum cup to a normal skin surface.

In the instances of death by drowning, nitrous oxide anesthesia, suffocation, and asphyxia by carbon monoxide, the anoxia was not accompanied by low atmospheric pressure. The same pattern of changes was present in these cases although the congestion and edema were less intense than when anoxia was combined with low atmospheric pressure.

### Low Atmospheric Pressure

Aviation at high altitude often causes collapse resembling surgical shock even though abundant oxygen is supplied. "The most dangerous aspects of severe reactions resulting from exposure to lowered barometric pressure are the latent period of from 1 to 6 hours which frequently precede the manifest appearance of clinical shock; and the consequent resistance to treatment of what may be, at this point, a full-blown vicious circle of peripheral circulatory insufficiency, tissue anoxia, and marked hemocentration." [39] Chambers from which the air was ex-

hausted, to simulate atmospheric conditions at various altitudes, were used to test the ability of aviators to withstand the effects of low pressure; oxygen was supplied as in actual high altitude flight. Fatalities occasionally resulted even under most careful supervision of such tests. I had opportunity to study the data and to determine the pathologic findings in 5 such cases for comparison with those of shock from other causes.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 95412 | 72 minutes at 38,000 ft. Death 10 hours later. |
| 100822 | One hour at 30,000 ft. Death 24 hours later. |
| 100893 | Discomfort and pain at 38,000 ft. Death 47 hours later. |
| 103767 | Discomfort and pain at 38,000 ft. Death 17 hours later. |
| 127451 | 20 minutes at 30,000 ft. Death 55 hours later. |

Since little is known of the mechanism causing shock from low atmospheric pressure, such cases are of special interest. Accordingly, condensed clinical and pathologic data from 2 representative cases are given.

*Case 100822*

After 1 hour at 30,000 ft. and while ascending to 38,000 ft., the subject gave up the test because of severe "bends" and "chokes." When taken out of the chamber he was very weak and sweating profusely. He was given oxygen by inhalation, and was placed under observation. The blood pressure was 140/104 mm. Hg; pulse, 90; red blood cells, 5,400,000; leukocytes, 23,000; hematocrit, 60. Roentgenograms of the chest showed pulmonary edema, recorded as moderately severe. Four hours later he was restless and vomited twice. The pulse was 140; the blood pressure, 120/80, later declining to 90/0. Plasma given intravenously caused no increase in the blood pressure; oxygen was continued. The next day he was weaker, restless, and cyanotic. Death occurred about 24 hours after the test.

At post-mortem examination, 750 cc. of serous fluid was found in each pleural cavity. The lungs, weighing 850 and 742 gm., were congested and markedly edematous. The heart weighed 410 gm. and showed no visible abnormalities. The spleen weighed 220 gm. and the liver, 2395 gm., with no abnormal features. Petechial hemorrhages were seen in the gastric mucosa and marked hemorrhages in the lining of the jejunum and ileum. There was congestion of the meninges. The kidneys, weighing 200 and 180 gm., were congested.

*Microscopic Examination.* The lungs showed intense capillovenous hyperemia and edema. The myocardium was edematous and the fibers showed parenchymatous changes. The spleen was ischemic. The liver showed degeneration, marked fatty changes, and central necrosis. There was intense hyperemia of the gastrointestinal mucosa. The cortical cells of the adrenals were vacuolated. The brain and meninges were hyperemic and edematous. The renal cortex and the medulla were moderately hyperemic. The tubular epithelium showed degenerative

changes which seemed more advanced in the upper than in the lower segments.

*Case 100893*

At a relative altitude of 38,000 ft., the subject became pale, the respirations were increased, and he complained of muscular pain and weakness. The altitude was reduced gradually and the test was discontinued. The pulse was 60; the blood pressure, 100/60 mm. Hg. The heart sounds were normal, the breath sounds were decreased, and the skin moist. He seemed drowsy and unable to talk coherently.

When placed in the hospital, the pulse was 80; the blood pressure, 110/80. Four hours later he seemed worse, irrational, and was thrashing about. Plasma, saline solution, and oxygen were given, and his general condition improved. The hematocrit at this time was 56.1; the white cells, 20,000. Three hours later his condition became grave, the hematocrit was 64.9, no pulse could be obtained; he was sweating and apparently in profound shock. The extremities were cold but the rectal temperature was 107.6°F.; on recheck 10 minutes later, this was 108.4° F. He was packed in ice and given plasma and saline solution. On the following day the general condition seemed improved but still critical. He was restless and the breathing was labored. The blood pressure was 122/80; hematocrit, 55. He was given plasma and saline solution continuously. On x-ray examination the cardiac shadow was not enlarged, but the pulmonary markings were increased and interpreted as vascular stasis. On the next day the respirations were easier, the blood pressure more nearly normal, and the temperature lower. The hematocrit was 50; white cells, 13,500. The urine showed a specific gravity of 1.017; albumin, 4 plus. He was seized with convulsions, the circulation failed, and death occurred 45 hours after admission to the hospital.

Post-mortem examination revealed marked hyperemia and edema of the lungs. These were described as solid in the posterior portions. The liver and brain were congested. No other gross abnormalities were recorded.

*Microscopic Examination.* The lungs were intensely hyperemic and edematous. There were areas of atelectasis and capillary hemorrhages (Fig. 16). The myocardium was edematous and degenerated. The splenic pulp was deficient in red blood cells. The liver was hyperemic; the cells showed advanced parenchymatous degeneration ranging to necrosis (Fig. 17). The adrenal cortex was hyperemic; the cortical cells were vacuolated. The brain showed widespread autolysis and a few perivascular hemorrhages. The renal parenchyma was hyperemic. The tubular epithelium was flat and the lumina were wide. Albuminous material was present in the glomerular spaces. The collecting tubules contained *dark, pigmented casts,* granular casts, and erythrocytes. The stroma was edematous (Figs. 18 and 19).

In each of the 5 cases, the gross and microscopic findings were essentially the same as in these 2. Shock from low atmospheric pressure presents some features not seen in shock from other causes: The body temperature was excessively high; there were convulsions and other signs of neurologic disturbances; extensive degeneration was found in

various parts of the central nervous system. In these particulars the syndrome resembles that of heat stroke, as will appear in the succeeding group. Several items were especially noteworthy in these cases: The concentration of the blood was high; in one case the hematocrit reading was 64.9. Other clinical signs of circulatory failure were marked. It is significant that the visceral findings were of the same pattern as in the other groups. The development of renal failure, with progressive retention of nitrogenous wastes, was rapid in case 100893. Pigmented casts and other changes in the kidneys were like those seen in the "crush syndrome" [40] although no transfusions nor sulfonamide therapy had been employed.

Several items are suggested as possible factors in the mechanism of shock from low atmospheric pressure. Low external pressure would contribute mechanically to the dilatation of minute vessels and to the leakage of fluid through their walls. This might derange fluid balance as seriously as would toxic or anoxic injury to endothelium; hence the hemoconcentration and circulatory deficiency. It has been suggested that low barometric pressure interferes directly with internal respiration even though abundant oxygen is supplied for breathing. Neither oxygen nor other gases under low pressure are absorbed readily by fluids. The hemoglobin of the erythrocytes is essentially a fluid; it may not absorb physiologic amounts of oxygen if the gaseous pressure is greatly decreased. Low external pressure may be directly injurious to the central nervous system. Extensive neurologic damage was found histologically in various parts of the brain. It is known that trauma, embolism, infection, or hemorrhage in some areas of the brain may produce the clinical syndrome of shock accompanied by hemoconcentration and characteristic visceral changes. The exact mechanism of these phenomena has not been shown and should be a fruitful subject for investigation by neurophysiologists.

Once the circulation and fluid balance are deranged, by any one of these mechanisms or by their combined effects, the resulting anoxia in the tissues will perpetuate the vicious cycle of secondary shock with its characteristic visceral and renal effects.

### Heat Stroke

Features of resemblance between heat stroke and secondary shock have been reported.[26] Drinker [41] noted sudden collapse and coma accompanied by vomiting and high fever, rapid pulse, and low blood pressure as the usual signs. He stated that the condition resembles surgical shock except for the elevation of temperature. Hill [42] ob-

served that at necropsy "the organs . . . . show capillary congestion, as in wound-shock."

Several authors [21, 22, 41, 43] include heat stroke among the conditions which may cause shock. Hartman [44, 45] observed manifestations of shock in cases of therapeutic hyperthermia resulting fatally. In one, the blood pressure sank to 68/20 at the end of the treatment; death came 20 hours later. He stated that the pathologic changes, both in human cases and in experimental animals, were typical of anoxia produced in other ways.

Twelve representative cases of heat stroke were selected in which death occurred after intervals varying from 25 minutes to 6 days; one case following therapeutic hyperthermia is included. Shock had been noted clinically in most cases.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 84012 | Fell unconscious during drill. Death 25 minutes later. |
| 97543 | Fell unconscious during hike. Death 3 hours later. |
| 96187 | Collapsed, comatose, after 25 mile hike. Death 6 hours later. |
| 112746 | Severe sunstroke while on duty. Death 6.5 hours later. |
| 85876 | Collapsed during "K.P." duty. Death 12 hours later. |
| 102099 | Fell unconscious while marching. Death 24 hours later. |
| 97148 | Collapsed during heat; shock, high temperature. Death 25 hours later. |
| 97556 | Collapsed during long distance run; oliguria. Death 34 hours later. |
| 113428 | Collapsed during heavy work in heat; oliguria, high blood nonprotein nitrogen. Death 36 hours later. |
| 96554 | Comatose after exposure to heat; oliguria, high nonprotein nitrogen. Death 3 days later. |
| 102705 | Heat exhaustion; oliguria, albuminuria, high nonprotein nitrogen. Death 6 days later. |
| 89594 | Collapse after therapeutic hyperthermia; anuria, high nonprotein nitrogen. Death 6 days later. |

The gross and microscopic changes found after death from heat stroke, therapeutic hyperthermia, and heat exhaustion were of the same types as described in the previous groups. Petechial hemorrhages were found in every case. The lungs were intensely hyperemic in 11, moderately hyperemic in one. Pulmonary edema was present in 9, atelectasis in 6, incipient pneumonia in 2. The spleen was engorged in 6. The kidneys were hyperemic in every instance. The myocardium was hyperemic in 7, edematous in 8, and showed degeneration in every case.

Parenchymatous degeneration and necrosis of the liver, kidneys, and heart were present regularly and were more extreme in degree than those seen in any other group of cases. It is known that postmortem changes develop most rapidly after death from heat stroke; perhaps this is due to the high body temperature which regularly pre-

ceded death. In 5 instances necropsies were performed in 1 to 3 hours after death; the changes mentioned were equally intense in these cases.

It is noteworthy that signs of renal disturbance occur incident to heat stroke. Albuminuria was noted in 4, oliguria in 4, and retention of nitrogenous wastes in 6. In many instances no laboratory studies on the urine and blood had been made. On microscopic examination, casts were found in the renal tubules in 6 of these cases (Figs. 25 and 26).

Subsequent to these studies, an extended survey on the pathology of heat stroke has been published by Malamud, Haymaker, and Custer [46] based upon material from 125 cases in the Army Institute of Pathology. Hyperthermia was a clinical feature in all but 2 of these. Clinical signs of shock were prominent; the ultimate outcome usually depended upon the degree of shock sustained. Pathologic changes in the central nervous system were conspicuous: progressive degeneration of neurons, congestion, edema, and petechial hemorrhages. The degeneration was attributed to excessive heat, the other changes to shock. "Evidences of acute circulatory failure, such as hemorrhage, edema, and vascular engorgement, were observed in virtually all cases regardless of the duration of illness." Serous effusions were recorded in 33 cases. "In virtually all cases there was intense vascular [pulmonary] congestion." This regularly was accompanied by edema; in no case was the weight of the lungs within normal limits. Some degree of pneumonia was found in 31 cases. Myocardial degeneration was frequent, ranging from irregular patches to large foci of necrosis. The kidneys regularly were hyperemic and above normal weight. The degree of parenchymatous change was less in cases of short duration. "Lower nephron nephrosis" was observed in 19 cases; this was severe in those who lived 35 hours or longer. The livers were congested and above normal weight; central lobular necrosis was seen in 12 of the cases which survived 31 hours or longer.

The recorded data support the belief that secondary shock is an important factor in death from heat stroke. Thermic deaths are accompanied by acute neurologic lesions. The mechanism by which such lesions cause shock has not been shown, but the presence of shock in such cases seems clearly established. The associated anoxia probably causes the parenchymatous degeneration and necrosis seen in the kidneys, liver, and heart. The clinical evidence of cerebral damage, the accompanying high fever, and the pathologic conditions found in the brain and in the viscera indicate that deaths from low atmospheric pressure and from heat stroke have certain features in common.

## Abdominal Emergencies

It has been observed that serious abdominal conditions may present the syndrome of shock. Instances are seen in perforations of the viscera, acute pancreatitis, intestinal obstruction as by volvulus or strangulated hernia, mesenteric thrombosis, and others. In several of these, such as acute hemorrhagic pancreatitis, perforation of the gall-bladder or of an ulcer, the onset of shock is immediate. In others, such as intestinal obstruction or peritonitis, the circulatory failure may be delayed somewhat. Ten such cases were surveyed:

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 101591 | Intestinal infarction, shock, anuria. Death 2.5 hours later. |
| 106742 | Intestinal obstruction, resection of gangrenous bowel. Death 24 hours later. |
| 108433 | Intestinal obstruction, operation for volvulus, anuria, albuminuria. |
| 114688 | Intestinal obstruction, operation. Collapse and death on 5th post-operative day. |
| 116017 | Intestinal obstruction, colostomy, followed by shock. |
| 117545 | Intestinal obstruction, operation. Death 2 days later. |
| 123485 | Intussusception, operation, anuria and albuminuria. Death 7 days later. |
| 111691 | Acute pancreatitis, oliguria, albuminuria. Collapse and death. |
| 130718 | Acute pancreatic necrosis of 2 days' duration, anuria, albuminuria, death. |
| 133910 | Acute pancreatitis, high nonprotein nitrogen, hemoconcentration. Death 5 days later. |

The findings in these cases were of exactly the same character as in shock from trauma and from other causes. The lungs were intensely congested and edematous in 7, moderately so in 3. Serous effusions were recorded in 7, petechiae in 6. The kidneys were markedly hyperemic in 3, moderately so in 7. The spleen was hyperemic in only 4. Parenchymatous degeneration ranging to necrosis was present in the liver and in the renal tubules in 6; post-mortem changes in 4 made the presence of these changes uncertain. The myocardium showed degenerative changes present in 7, absent in one; in 2 cases sections of heart were lacking.

In 6 cases, surgical relief had been attempted. These might have been included appropriately in the first group. They represent shock resulting from a combination of factors, including anesthesia, surgical trauma, local loss of blood and fluid, and the grave condition of disease which made operation necessary. In the remaining 4 instances, the disease itself led to circulatory failure and death. It is significant that evidences of renal disturbance were seen clinically in several cases; in others no laboratory tests were recorded.

## Miscellaneous

Under the heading of miscellaneous are included deaths from various causes including anaphylaxis, allergic reactions, and sudden death from unexplained causes. Sometimes acute infection or poison was shown as the cause of death by post-mortem examinations; such cases are not included in this group. Ten representative instances of sudden or unexplained death were studied.

| *A.I.P. Accession Number* | *Conditions* |
|---|---|
| 86556 | Child died in bed; no previous illness, lymphoid hyperplasia found. |
| 104029 | Shock reaction after injection of nupercaine. Death 1 hour later. |
| 105156 | Received typhoid and cholera vaccine, collapse. Death 20 minutes later. |
| 109249 | Sudden death, cause undetermined. |
| 116783 | Typhoid vaccine intravenously, treatment for arthritis. Death 23 hours later. |
| 116840 | Received typhus vaccine, no immediate reaction. Died during night. |
| 121120 | Shock reaction to diphtheria antitoxin. Death 14 hours later. |
| 128537 | Sudden death, cause undetermined. |
| 131480 | Sudden death, cause undetermined. |
| 145523 | Sudden death, cause undetermined. |

In each instance, pulmonary hyperemia and edema were marked; petechiae were present in 9; serous effusions were found in 2. The kidneys were hyperemic in 9, the liver in 7, the spleen in 5. The liver and kidneys showed marked degeneration, ranging to necrosis in 7 cases; post-mortem changes in 3 made degeneration uncertain.

It is known that the visceral findings after anaphylactic death in man and in some animals often are identical with those of secondary shock. Those dying of trivial or insufficient causes often are shown to have a subnormal amount of adrenal cortical tissue. This has been suggested as an explanation for status lymphaticus. The adrenals were hypoplastic in cases 86556, 105156, 109249, and 145523; in 2 other cases no sections of the adrenals were available. Since the adrenal cortical hormone is an important factor in the "alarm reaction" (Selye [47]), deficiency of it may be a factor in deaths without apparent adequate cause.

## Renal Pathology

It has been observed by many authors that shock from diverse causes was accompanied by an increase in the nonprotein nitrogen of the blood. This was thought by Whipple, Smith, and Belt [48] to arise from two sources: disintegration of the body's own protein, and deficient elimination of nitrogenous wastes by the kidneys.

Evidence of renal effects accumulated as the phenomena of shock

were studied, until by 1941 their importance appeared so paramount that they were included among characteristic functional disturbances in a definition of shock.[26] That evidence is too extensive for detailed review here. It included Bell's [49] report on "clinical acute nephritis" as distinct from glomerulonephritis, the contributions of Bordley [28] and Daniels, Leonard, and Holtzman [50] on renal effects of transfusions, Lambret and Driessens' [51] studies on physiologic disturbances accompanying surgical shock, Helwig and Schutz' [52] and Boyce and McFetridge's [53] reports on uremia associated with so-called "liver death," Christophe's [54] observations on "acute nephritis" as a regular feature of burns, both clinical and experimental, Bywaters' [40] observation on uremia from compression of limbs, and the review of Jeghers and Bakst [55] on extrarenal uremia.

The reported conditions under which acute uremia developed, independent of glomerulonephritis, included intestinal obstruction, toxic jaundice, "hepatorenal syndrome," extensive surgery, intestinal perforation, poisoning with phenobarbital, cincophen and other drugs, streptococcal cellulitis and other severe infections, diabetic coma and other metabolic intoxications, anaphylactic reactions, transfusion with incompatible blood, acute pancreatitis, cerebral lesions such as abscess or hemorrhage, sublethal shock from trauma as in the "crush syndrome," and burns. In many instances it was recognized that the renal deficiency was associated with shock. Some found hemoconcentration of value in differentiating this type of uremia from other types.

In the cases summarized here, renal deficiency was prominent when a state of sublethal shock had persisted for 2 days or longer. Instances of this were seen after accidental trauma; gunshot wounds; extensive surgical operations; transfusion reactions; burns; poisoning with arsenicals, mercury, barbiturates and with phosphorus; tularemia, malaria, and other infections; shock from low atmospheric pressure, therapeutic hyperthermia, and from heat stroke, intestinal obstruction, and after miscellaneous causes. In many of these instances, neither transfusion of blood nor chemotherapy, as with sulfonamide drugs, had been employed.

The pathologic features set forth in previous reports do not differ in essential particulars from those described in the groups of cases summarized here. Grossly, the kidneys may be of normal size or moderately enlarged; usually they are soft, indicating edema. The capsule often strips with unusual ease; the stellate veins in the cortical surfaces are engorged. The color varies, depending upon relative degree of congestion and of parenchymatous changes. When congestion pre-

dominates, the color is deep red; degenerative changes produce varying degrees of pallor; cloudy swelling usually is mentioned. Red streaks are seen in the medulla and the cortical markings frequently are obscured. Sometimes petechiae are described in the parenchyma or in the pelvic lining.

The microscopic picture shows varying degrees of changes within a regular pattern. Usually hyperemia of the glomerular tufts and of the intertubular capillaries and venules is conspicuous. Sometimes capillary hemorrhages are seen. Red streaks in the medulla result from patchy dilatation of groups of venules which parallel the straight tubules. The epithelium of the convoluted tubules shows varying degrees of acute degeneration. The cytoplasm is granular; more marked degeneration is indicated by vacuolation of the cytoplasm or disintegration of the cells. The nuclei may be hyperchromic, hypochromic, or karyolytic.

Hyaline droplets in the cytoplasm are seen often when degeneration is marked; the lumina may contain globules or masses of this deeply acidophilic material when the cells containing it have disintegrated. Because of their dense staining, these may be mistaken for red blood cells or their pigment. Sometimes these changes are more marked in the upper segment, sometimes in the lower, but usually all portions of the convoluted tubules are affected. Plugs of débris often occur in the lumina of the lower segments as such débris, originating in the upper portion of the nephron, collects in the narrow lumina of the lower portion. Hyaline, granular, and sometimes pigmented casts, débris, erythrocytes, and the nuclei of the degenerated epithelial cells are usually found in the collecting tubules. Edema is a varying feature usually more marked in the medullary than in the cortical zones.

Two recent reports have dealt with renal changes associated with shock. Herbut [56] confirmed the syndrome of uremia noted by others, consisting of oliguria, albuminuria, casts, erythrocytes and débris in the urine, and progressive azotemia. These features developed incident to acute or to sublethal shock from diverse causes. Cases were described resulting from transfusion reactions, bile peritonitis, ulcerative enteritis, abscess, burns, extensive necrosis of neoplasm, obstructive jaundice, sulfonamide therapy, mercuric and arsenical poisoning. The gross features of the kidneys were as described above. The microscopic characteristics were hyperemia and edema of the glomeruli and interstitial tissue; severe degeneration ranging to necrosis of the tubular epithelium, most marked in *proximal* portions; granular eosino-

philic material in the lumina of both proximal and distal portions; and various types of casts in the distal and collecting tubules.

Lucké [57] reported on "lower nephron nephrosis," based upon records and material from numerous cases in the Army Institute of Pathology. These represented battle wounds, crushing injuries, abdominal operations, burns, transfusion reactions, sulfonamide intoxication (combined with infections, trauma, etc.), heat stroke, malaria, various poisons, hemolytic anemia, and miscellaneous conditions such as eclampsia, pancreatitis, and shock from various causes. Shock and vomiting were the two conditions most commonly associated. The clinical manifestations were oliguria, dark or bloody urine containing heme pigment, albumin and casts, progressive azotemia, hypertension, edema, and uremia. The gross features of the kidneys were essentially as given above. Microscopically, acute degeneration ranging to necrosis was found regularly. This involved chiefly the *lower* segments of the convoluted tubules. Protein material was present in the capsular spaces. The tubular lumina contained pigmented and nonpigmented casts and cellular débris.

These changes were as observed by others except for one particular: Lucké [57] found degeneration and necrosis sharply limited to the lower portion of the nephron. Others have described such changes in similar cases as involving all parts of the convoluted tubules. My own observations coincide with the latter findings (Figs. 4, 7, 18, 20, 24, and 25).

## Summary

In the cases studied, the pattern of visceral changes characteristic of secondary shock was found after death from various forms of acute disease. These included the combined effects of trauma, hemorrhages, infection, anesthesia, transfusion, and surgery; burns, poisoning with various drugs and chemicals, severe infections, anoxia from sundry causes, low atmospheric pressure, heat stroke, abdominal emergencies such as intestinal obstruction and pancreatitis, and sudden death from anaphylaxis and other causes.

The pattern of changes indicative of endothelial damage included dilatation and engorgement of capillaries and venules in the thoracic, abdominal, and cranial viscera, petechiae in serous and mucous surfaces and within parenchymatous tissues, and edema of soft viscera. Two modes of origin are suggested for the acute degeneration ranging to necrosis which was seen in the kidneys, liver, and myocardium: (1) The same injurious agents which damage endothelium and cause

capillary dilatation and permeability likewise may cause parenchymatous degeneration and necrosis; this is seen in the effects of various poisons and bacterial products. (2) The same effects resulted from uncomplicated anoxia, indicating that endothelial, renal, hepatic, and myocardial cells are delicately susceptible to lack of oxygen. It seems probable that the parenchymatous changes, found after death from shock, result from a combination of these two mechanisms.

Pulmonary hyperemia and edema occurred in a high percentage of cases. When death was delayed 48 hours or longer, the development of secondary or terminal pneumonia often occurred. Lungs in which the circulation is impaired and the spaces filled with albuminous fluid will almost certainly develop secondary pneumonia if neither death nor recovery occurs soon.[24] This was exemplified in numerous instances when shock persisted for several days in a sublethal degree.

Some degree of atelectasis was found in almost one-half of the cases. No previous comment has been made on this feature; its origin and relationship to the mechanism of shock are not apparent. Hemorrhagic infarcts, suggesting embolism, were found in several cases.

Renal functional deficiency was a prominent feature of sublethal shock lasting several days. This was manifested clinically by oliguria, albuminuria, hematuria, and by progressive retention of nitrogenous wastes sometimes terminating in uremia. The pathologic renal features included hyperemia and edema; parenchymatous degeneration and necrosis of tubular epithelium, with desquamation into the lumina; débris; hyaline, granular, and sometimes pigmented casts. These features correspond to the picture of *acute tubular* or *parenchymatous* nephritis described by internists and pathologists of the preceding generation.

Hepatic degeneration and necrosis were regular features, but their degree and distribution were inconstant. Degeneration tended to be diffuse and the degree varied from granular cytoplasm and vesicular nuclei to necrosis. Absence of nuclei, pyknosis, and disintegrating cells were the criteria for necrosis. Frequently this involved only scattered groups of cells; when the groups were larger, focal necrosis was seen. Occasionally, as after burns, heat stroke, or death from low atmospheric pressure, extensive necrosis involved the centers of the lobules and resembled that produced by poisons.

Degeneration of the myocardium was less constant than that of the kidneys and liver; its degree varied. Often the cross striations were obliterated, the substance of the fibers was granular or lumpy rather than of uniform density, and the nuclei were swollen or distorted.

Apparent liquefaction of the fibers, basophilic staining reaction, or marked transverse fragmentation indicated severe degeneration.

Splenic changes were not consistent; in some instances the spleen was enlarged and engorged, in others it was flabby, contracted, and ischemic. In a number of sections it was noted that the lymphoid follicles were small, inconspicuous, and contained few lymphoid cells. This apparently did not pertain to any one type of causative condition; it was observed in several of the groups. There was no evidence to indicate whether its origin is related to shock. The histologic preparations did not always include lymph nodes; when present they were regularly edematous and occasionally hyperemic.

Hyperemia, edema, and petechiae often were found in the mucosa of the stomach and small bowel. This feature was not regular. Petechial hemorrhages in mucous and serous surfaces and in the substance of organs were present in the majority of cases. Their highest frequency was after poisoning, burns, anoxia, and after heat stroke. This indicates that lack of oxygen is an important factor in endothelial damage.

Sections of adrenal were available in about one-half of the cases. In the majority of these the cells of the zona fasciculata appeared abnormally vacuolated. In some, the cells were disintegrated and in a few instances there was focal necrosis. These observations are in accord with those of Selye [47] who emphasized depletion of the adrenal cortical cells as a feature of shock. In a few cases of fulminating meningoccal meningitis, the adrenals were diffusely hemorrhagic and almost totally necrotic. This was not seen in any instance of shock from other causes.

Other ductless glands were not regularly included among the sections available. In several instances the pituitary body was markedly hyperemic and the cells of the anterior lobe appeared degenerated. Occasionally the thyroid and thymus were hyperemic.

This study corroborates the occurrence of secondary shock in other conditions than severe trauma, burns, and after extensive surgery. It develops in sundry conditions which may cause either capillary atony or anoxia. The resulting circulatory effects in the viscera indicate widespread capillary damage. The changes varied somewhat in degree but the pattern was consistent.

The parenchymatous effects may be ascribed in part to the injurious agent itself, in part to anoxia. The severe effects seen regularly in the renal tubular epithelium probably are related to the anuria and other evidences of renal functional deficiency which accompany shock.

It appears that secondary shock, like other conditions of disease, is accompanied by distinctive morphologic changes which are related to its mechanism of origin and to the associated functional disturbances.

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## DESCRIPTION OF PLATES

### PLATE 52

FIG. 1. Army Institute of Pathology accession no. 114945, multiple gunshot wounds. Lung showing intense capillovenous hyperemia, edema, capillary hemorrhages, and slight leukocytic infiltration. × 160. (A.I.P. negative no. 86843.)

FIG. 2. From the same case as Figure 1. Adrenal cortex showing focal necrosis. × 145. (Neg. 89858.)

FIG. 3. A.I.P. acc. 102060, shock following surgery, death after 4 days. Liver showing acute degeneration and necrosis. × 220. (Neg. 89849.)

FIG. 4. From the same case as Figure 3. Renal cortex showing albuminous matter in the capsular space, degeneration and necrosis of tubular epithelium affecting both upper and lower segments, and débris in the lumina. × 250. (Neg. 86826.)

FIG. 5. Renal medulla from the same section as Figure 4. Of note are masses of desquamated renal epithelium filling the lumina of the collecting tubules. Many contain erythrocytes. × 160. (Neg. 86836.)

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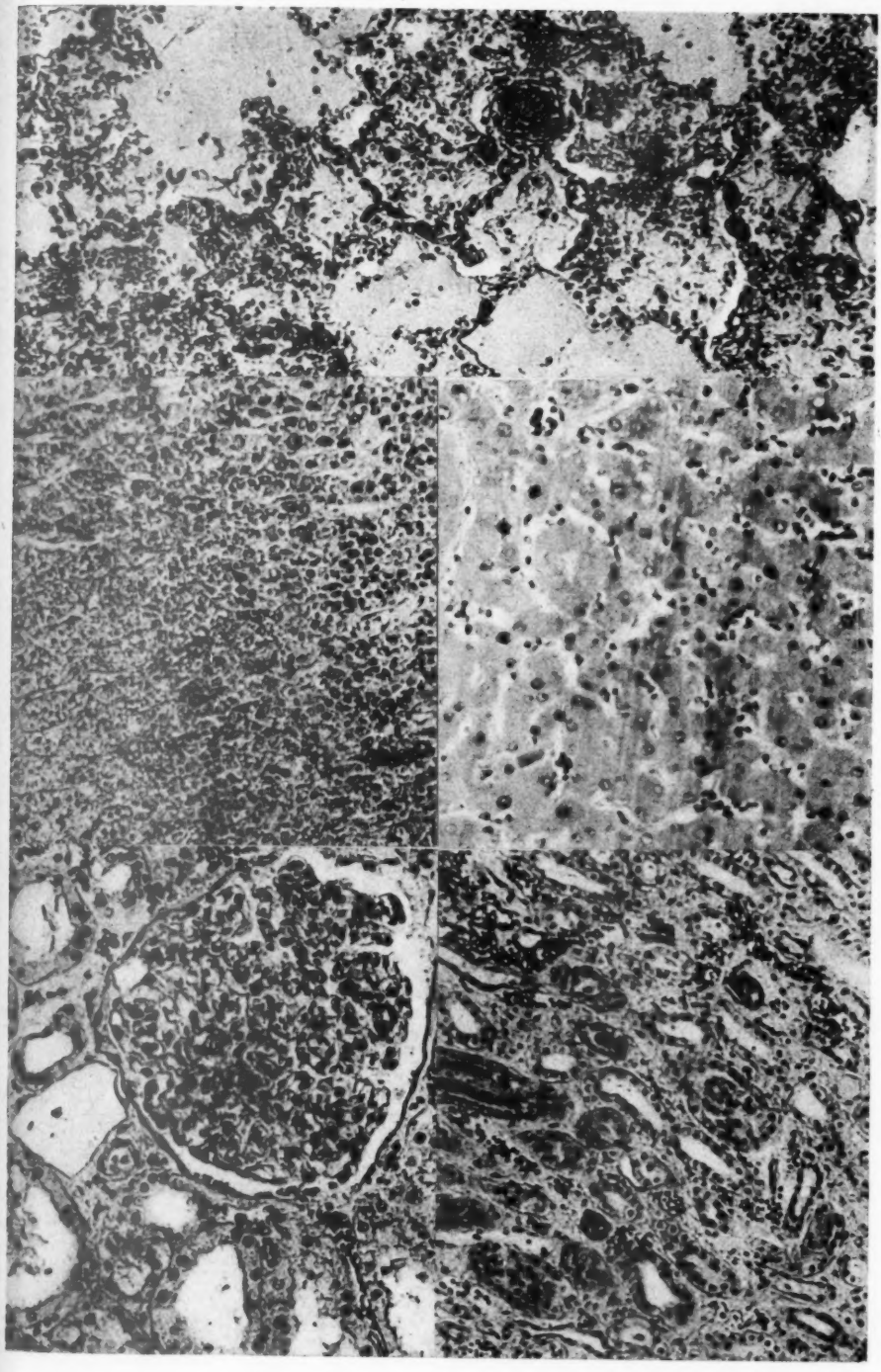



Moon

Pathology of Secondary Shock

PLATE 53

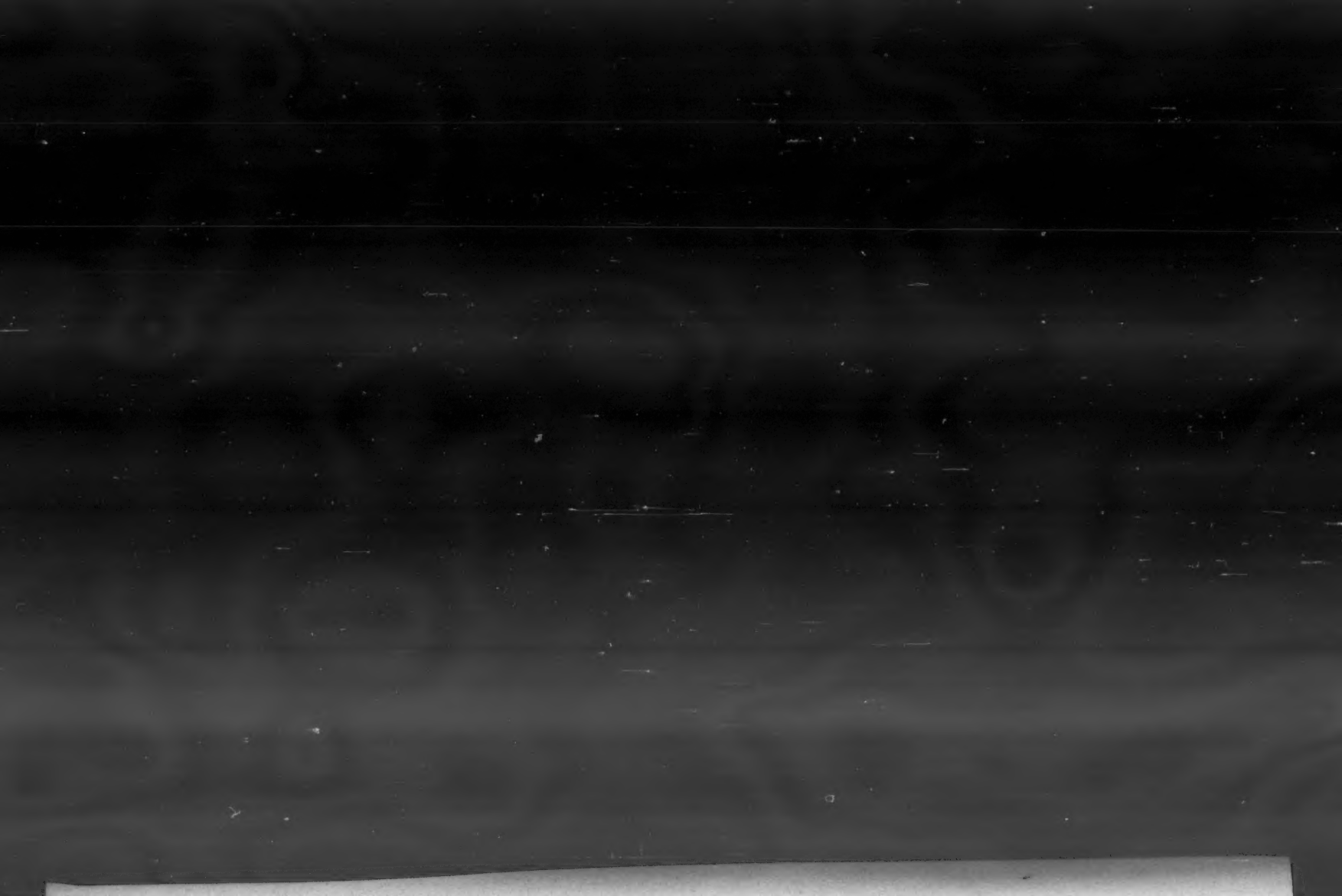
FIG. 6. A.I.P. acc. 114072, death 8 hours after burns of the skin. Lung showing intense hyperemia, edema, and capillary hemorrhages. × 145. (Neg. 68819.)

FIG. 7. A.I.P. acc. 118506, death 8 days after burns, post-mortem interval of 1 hour. Renal cortex showing acute degeneration, necrosis of individual cells, casts and débris in lumina, and albuminous matter in capsular space. × 230. (Neg. 86813.)

FIG. 8. Renal medulla from the same section as Figure 2. Of note are hyperemia, casts, and débris in the lumina. × 230. (Neg. 86821.)

FIG. 9. Liver from the same case as Figure 8. Marked acute degeneration and necrosis. × 280. (Neg. 89850.)

FIG. 10. From the same case as Figures 3 to 5. Lung showing hyperemia, edema, and beginning pneumonia. × 160. (Neg. 86824.)



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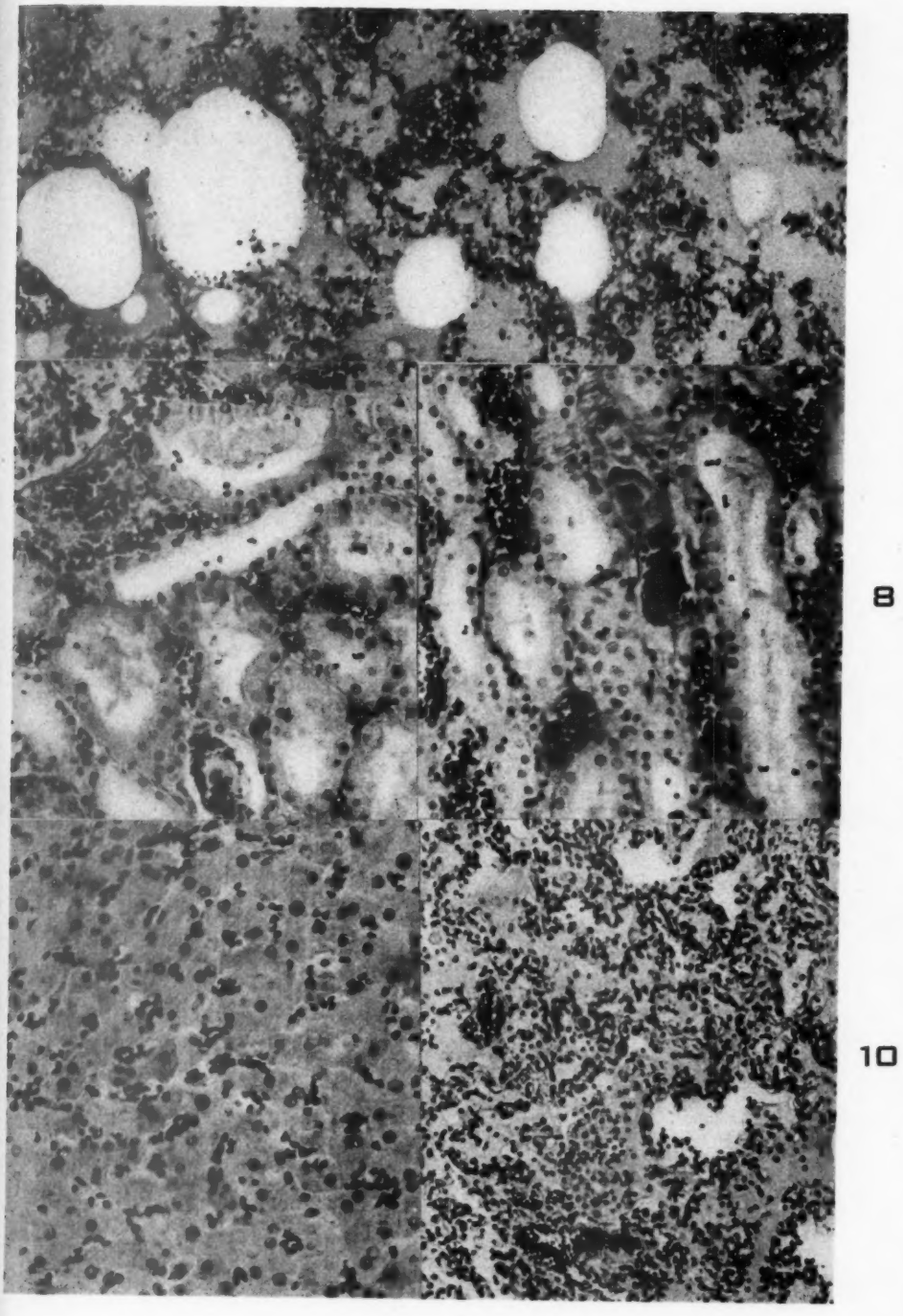


Moon

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## Plate 54

Fig. 11. A.I.P. acc. 111925, shock reaction to mapharsen. Lung showing hyperemia, edema, and early pneumonia. × 130. (Neg. 89855.)

Fig. 12. From the same case as Figure 11. Renal cortex, showing degeneration and necrosis of the tubular epithelium, casts, hyperemia, and edema. × 145. (Neg. 89856.)

Fig. 13. From the same case as Figures 11 and 12. Renal medulla, showing granular and pigmented casts and erythrocytes in the lumina. Edema and hyperemia are seen also. × 175. (Neg. 86829.)

Fig. 14. A.I.P. acc. 116989, poisoning with phenobarbital. Myocardium showing degeneration and fragmentation. × 160. (Neg. 89864.)

Fig. 15. A.I.P. acc. 118865, fulminating meningitis. Lung showing hyperemia, edema, and slight atelectasis. × 120. (Neg. 89863.)

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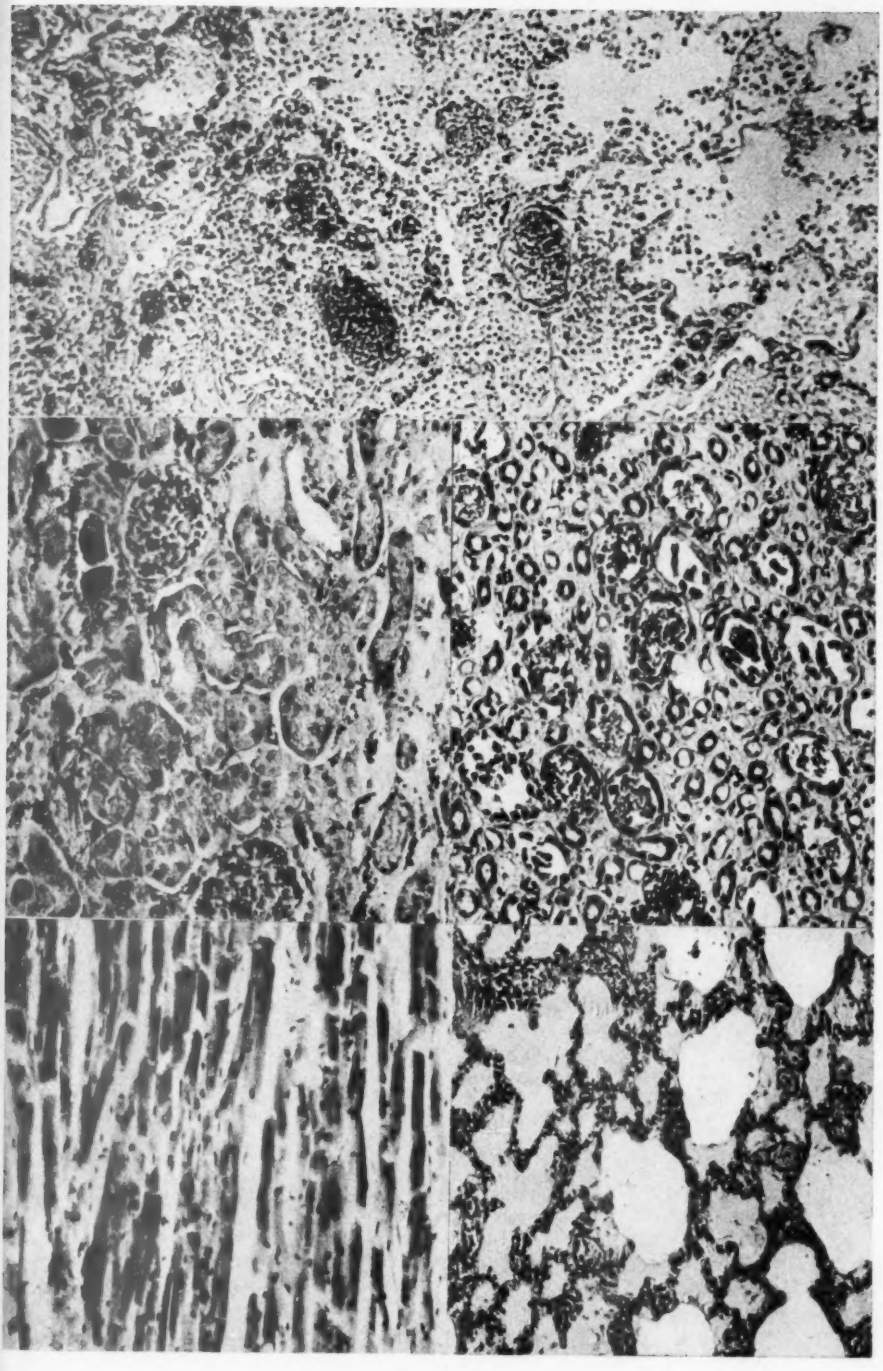


Moon Pathology of Secondary Shock

## Plate 55

Fig. 16. A.I.P. acc. 100893, shock from low atmospheric pressure in experimental test. Intense hyperemia, edema, and slight leukocytic infiltration are shown in this lung. × 145. (Neg. 86838.)

Fig. 17. From the same case as Figure 16. Liver showing acute degeneration, necrosis of individual cells, and edema. × 230. (Neg. 86834.)

Fig. 18. From the same case as Figures 16 and 17. Renal cortex showing low cuboidal epithelium, débris and casts in wide lumina, degeneration and necrosis of epithelium, and albuminous matter in capsular space. × 250. (Neg. 86835.)

Fig. 19. From the same case as Figures 16 to 18. Renal medulla, same section as Figure 18. Of note are the granular, amorphous, pigmented, and epithelial casts. No transfusion of blood and no drug therapy had been given. × 250. (Neg. 86817.)

Fig. 20. A.I.P. acc. 127451, shock from high-altitude aviation. Renal cortex showing marked degeneration and necrosis, desquamation, débris in lumina, and in the capsular space. × 280. (Neg. 86812.)

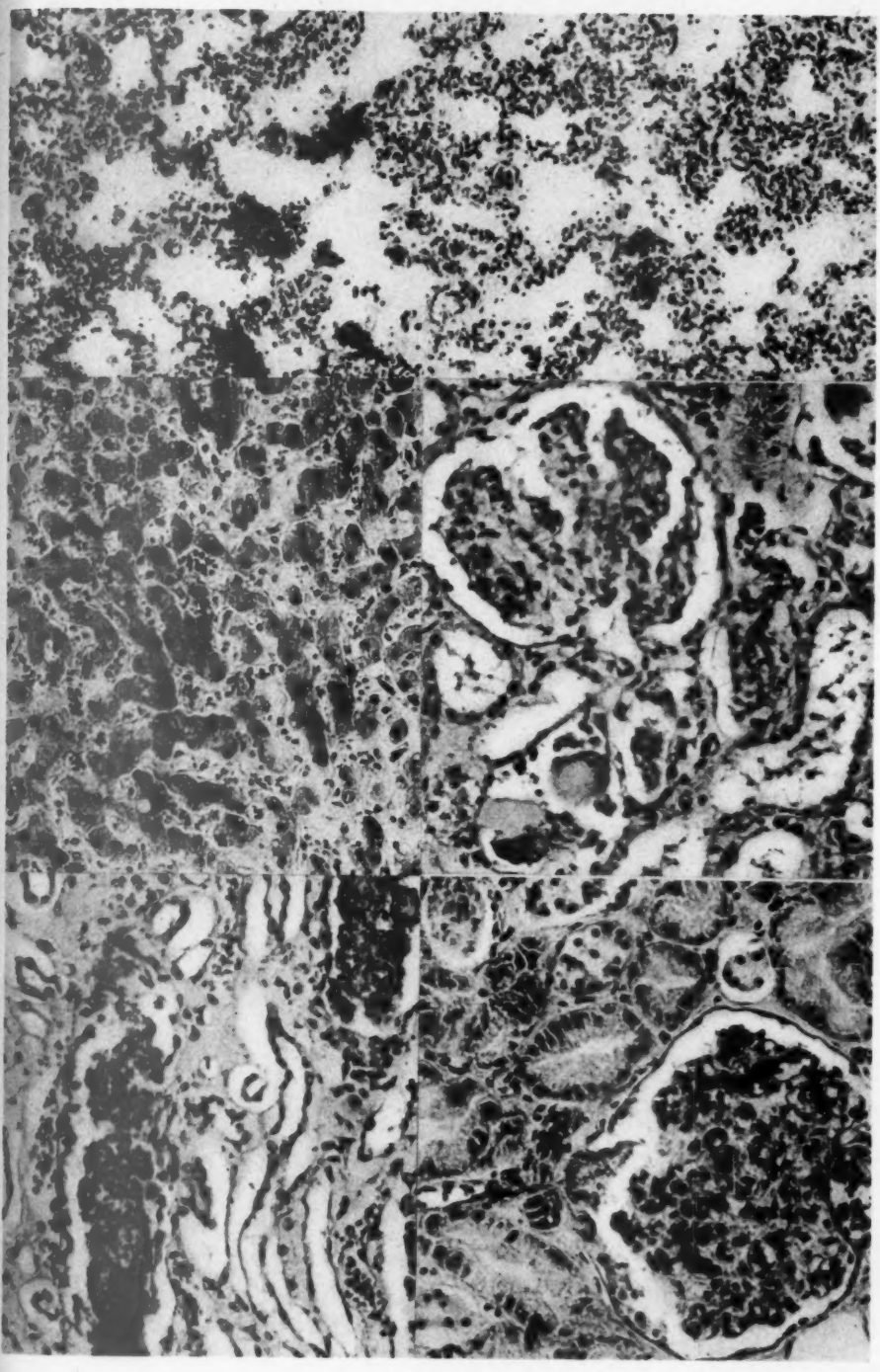


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PLATE 56

FIG. 21. A.I.P. acc. 128485, intestinal obstruction and surgery. Lung showing hyperemia and edema. × 175. (Neg. 86840.)

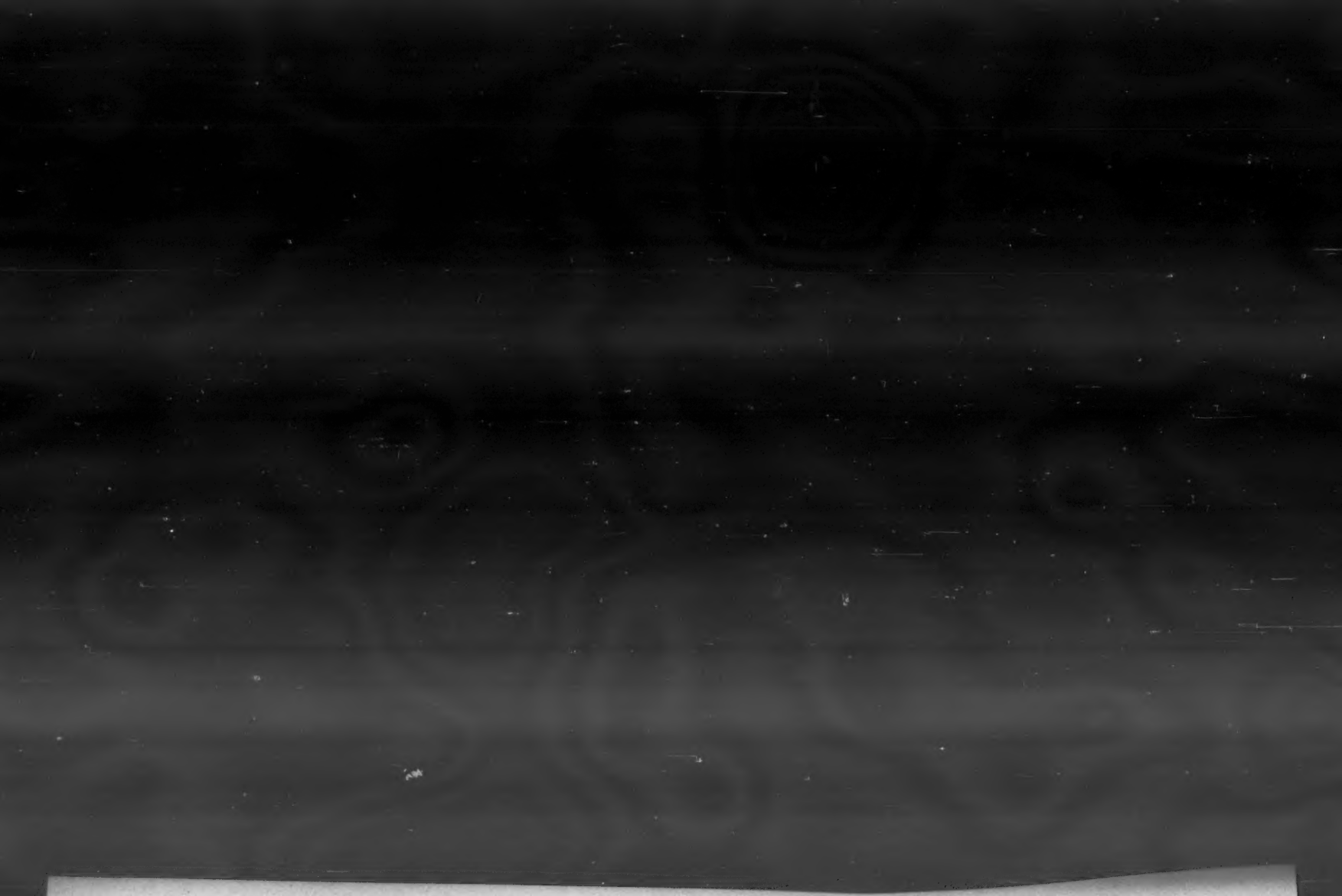
FIG. 22. A.I.P. acc. 128537, sudden death, cause undetermined. Myocardium showing marked degeneration, fragmentation, necrosis, and slight edema. × 180. (Neg. 86833.)

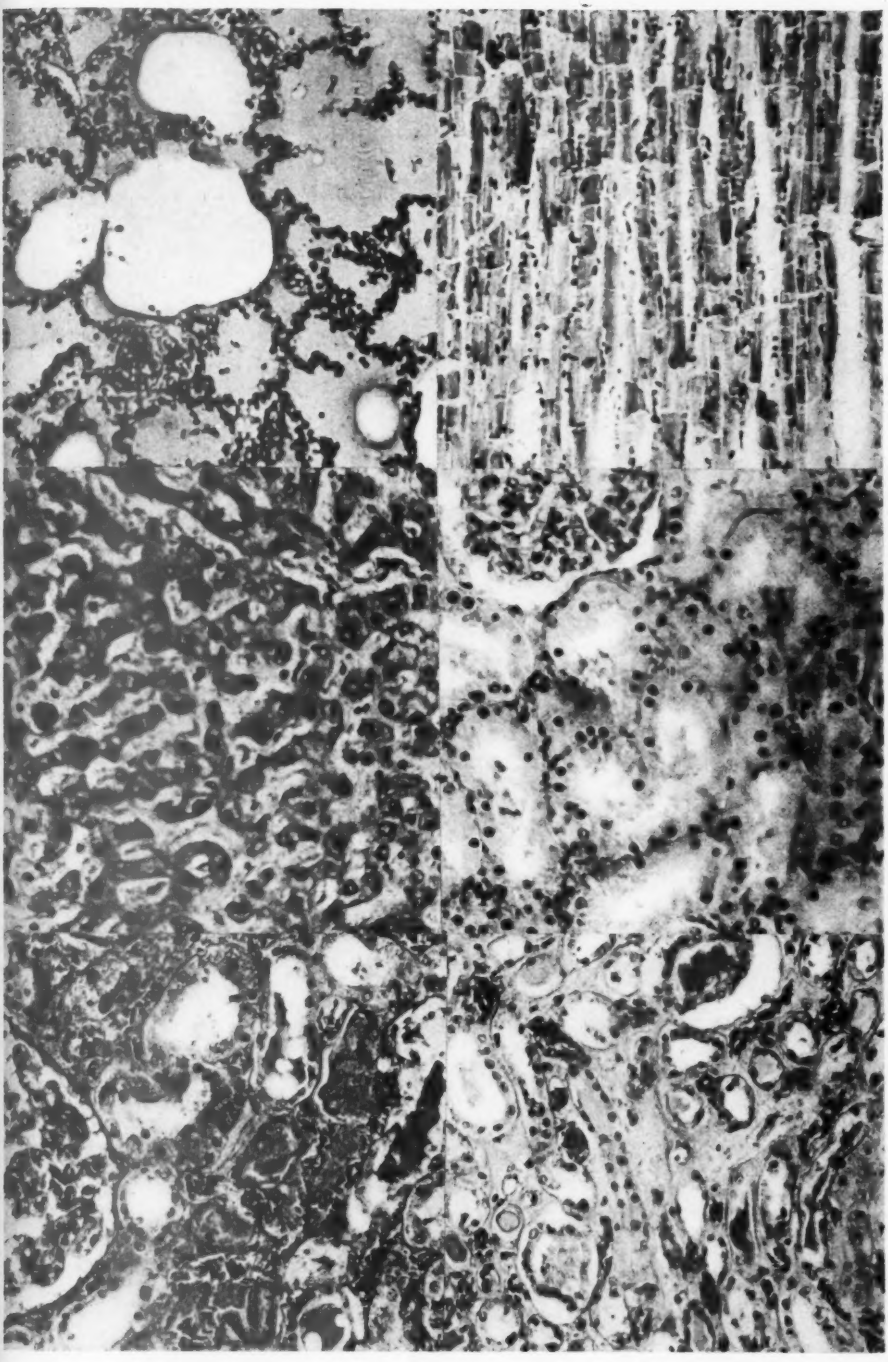
FIG. 23. A.I.P. acc. 83172, death from lack of oxygen. Liver showing acute degeneration, early necrosis, and marked edema. × 230. (Neg. 86810.)

FIG. 24. A.I.P. acc. 111237, lack of oxygen in high-altitude aviation. Renal cortex showing acute degeneration of epithelium, many pyknotic nuclei, and albuminous matter in the capsular space. × 300. (Neg. 86831.)

FIG. 25. A.I.P. acc. 113428, heat stroke fatal in 36 hours. Renal cortex showing extensive necrosis, casts, and débris in the lumina. × 230. (Neg. 86825.)

FIG. 26. Same section as Figure 25. Renal medulla. Of note are the granular, hyaline, amorphous, and pigmented casts. This patient had received no transfusion of blood nor drug therapy. × 230. (Neg. 86822.)






Moon Pathology of Secondary Shock

# STUDIES ON THE MECHANISM OF ACTION OF THE NITROGEN AND SULFUR MUSTARDS IN VIVO *

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The biologic actions of the sulfur and nitrogen mustards have been reviewed recently,[1] and detailed descriptions of the toxicologic and pathologic effects of these compounds have been submitted for publication.[2,3] At $LD_{50}$ doses, via any route of administration, these compounds produce a characteristic toxicologic *systemic* effect consisting of anorexia, weight loss, diarrhea, and leukopenia, terminating in death 3 to 5 days after injection. Clinical and pathologic examinations show that lymphopenia appears within 6 to 12 hours after injection and is associated with lymphocytic destruction and involution of the lymphatic tissue, thymus, and spleen. This is followed by progressive decrease in granulocytes to the low level of 200 to 300 cells per cmm. within 3 days after injection, associated with aplasia of the bone marrow; and diarrhea beginning 2 days after injection, associated with demonstrable desquamative degenerative changes in the intestinal mucosa.[3]

This report consists of a series of experiments designed to elucidate the mechanism whereby these effects are produced.

## THE "ALARM REACTION" AND ITS RELATION TO THE LYMPHOCYTOTOXIC ACTION OF NITROGEN MUSTARD

The systemic intoxication produced by the mustard compounds in rats resembles in many respects a pattern of organic changes that has been termed by Selye [4,5] the "alarm reaction."† This pattern is alleged to follow the subjection of normal rats to a wide variety of unrelated, sublethal injurious procedures, *e.g.*, cold, traumatic injury, excessive muscular exercise, spinal shock, acute infection, and injections of formaldehyde, morphine, atropine, and adrenalin.[5] According to Selye's description, this reaction consists of *"a rapid decrease* in the *size of the thymus, spleen, lymph glands* and liver; disappearance of fat tissue; edema formation, especially in the thymus and loose retroperi-

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† "An alarm reaction develops after the administration of any drug administered in sublethal doses, unless the specific pharmacological actions of the drug exert such a violent selective action on vital centers (heart, respiratory center, etc.) that death ensues as a result of this selective action before any marked general damage occurs." Selye.[5]

toneal connective tissue; accumulation of pleural and peritoneal transudate; *loss of muscular tone;* fall of body temperature; *formation of acute erosions in the digestive tract,* particularly in the stomach, *small intestine* and appendix; loss of cortical lipoids and chromaffin substance from the adrenals; and sometimes hyperemia of the skin, exophthalmos, increased lachrymation and salivation. In very severe cases, focal necrosis of the liver and dense clouding of the crystalline lens may be observed." (The italics are ours and indicate features which are common in animals intoxicated with the mustard compounds.) Marked leukocytosis with lymphopenia usually occurs within 48 hours with the "alarm reaction," but leukopenia develops if the treatment is severe.[6]

Microscopically, the lymphoid tissue in the "alarm reaction" presents a picture of massive lymphocytic necrosis and fragmentation with contraction of the organ and increase in reticular elements and frequent hemorrhages.[7] The adrenal cortex becomes hyperplastic with a loss of lipoid granules, while the medulla loses its chromaffin granules and vacuoles appear in the periphery of its cells; if the "alarm reaction" is severe the cells undergo necrosis. Other changes occur in the pancreas, the gastric and intestinal mucosa, and the liver.

Selye postulated that the "alarm reaction" is due to a common substance released in the body by all varieties of noxious stimuli. In normal rats this substance induces adrenal hypertrophy and hypersecretion, which in turn causes involution of the lymphatic tissue, for in the absence of the adrenal glands the "alarm reaction" does not induce lymphatic atrophy.[5] Selye stated that "So far, the only substances with which we have been able to cause thymus involution in the adrenalectomized rat are cortical extract and estrone."

It is to be assumed under Selye's hypothesis that the mustard compounds, because of their severe and prolonged intoxicating effect, must invoke some degree of the "alarm reaction." It is necessary to demonstrate, however, that the specific pathologic effects attributable to the mustard compounds are not indirectly due to the "alarm reaction." In the case of the lymphatic tissue, this was shown in adrenalectomized rats as follows.

### THE EFFECT OF METHYL-BIS (β-CHLOROETHYL) AMINE HYDROCHLORIDE ($HN_2$·HCL)* IN ADRENALECTOMIZED RATS

Before a definitive experiment could be performed, the toxicity of $HN_2$·HCl in adrenalectomized rats was determined. Such rats, ade-

* $HN_2$ is the official designation of this nitrogen mustard compound; $HN_2$·HCl is the hydrochloride salt.

quately maintained on salt, are unduly sensitive to $HN_2$ intoxication both in regard to dosage and to the acceleration of intoxication; 10 mg. per kg. of the HCl salt given subcutaneously are fatal in 3 to 6 hours after injection, whereas most normal rats survive for 72 hours; 3 mg. per kg. cause death in 48 to 60 hours, instead of 78 to 100 hours for the controls; and 1 mg. per kg. ($\frac{1}{2}$ $LD_{50}$) is fatal in 3 to 4 days whereas controls survive such a dose without difficulty. A dose of 3 mg. per kg. was used in the experiment reported.

### *Methods*

Fifty-six adult female rats, weighing from 150 to 210 gm., were offered 1 per cent saline solution in tap water beginning 24 hours before operation and during the course of the experiment. Four groups were employed.

*Group I.* (*8 Sham-operated Control Rats.*) The rats were placed on limited rations so that they suffered a slight progressive weight loss. It is difficult to prepare a control group whose food intake and weight loss will parallel that seen after $HN_2$ since changes in fluid balance and gastric distention with food complicate the picture in the intoxicated rat. It was felt, however, that animals on decreased food intake for a short period would furnish a more adequate control than normal well fed rats.

*Group II.* (*10 Adrenalectomized Rats.*) These were fed *ad libitum.*

*Group III.* (*18 Sham-operated Rats Receiving 3 mg. per kg. of $HN_2$·HCl Subcutaneously 24 Hours after Operation.*) These animals were fed *ad libitum.*

*Group IV.* (*20 Adrenalectomized Rats Receiving 3 mg. per kg. of $HN_2$·HCl Subcutaneously 24 Hours after Operation.*) These animals were fed *ad libitum.*

Operations were performed under ether anesthesia. Twenty-four hours after operation 3 mg. per kg. of $HN_2$·HCl in 0.8 cc. of saline solution were given to each animal in the appropriate groups. This represents an $LD_{99}$ dose in normal animals, the $LD_{50}$ being 1.9 mg. per kg. The rats were weighed daily. Sacrifices were performed by exsanguination from the abdominal aorta under ether anesthesia. Blood counts and smears were taken from the freely flowing aortic blood at autopsy. Organs were removed, blotted to free them of blood, and weighed to the nearest milligram on a Roller-Smith torsion balance. The lungs were weighed in tared beakers and then dried for 18 hours at 88 to 90° C. The data on the weight of the organs are insufficient to justify corrections for body weight or statistical analysis; only the averages and the extreme range of weights for each organ are given.

TABLE I

*Effects of 3 mg. per kg. of HN2·HCl Methyl-bis (β-chloroethyl) Amine Hydrochloride on the Normal and Adrenalectomized Rat; Body and Organ Weights at Time of Sacrifice*

| | Controls | | Injected with 3 mg. per kg. of HN2·HCl | | | | |
|---|---|---|---|---|---|---|---|
| | | | Group III Sham-operated | | | Group IV Adrenalectomized | |
| | | | Time of sacrifice after injection, in hours | | | | |
| | Group I Sham-operated, diet restricted | Group II Adrenalectomized | 24 | 48 | 72 | 24 | 48 |
| No. of rats | 8 | 10 | 5 | 4 | 8 | 4 | 5 |
| Pre-treatment wgt. (gm.) | 193 (182–200) | 182 (170–195) | 184 (170–200) | 184 (172–200) | 195 (185–208) | 184 (165–200) | 190 (175–200) |
| Aver. wgt. at sacrifice (gm.) | 178 (169–192) | 181 (168–208) | 172 (164–184) | 163 (150–178) | 162 (150–185) | 175 (159–197) | 171 (142–192) |
| Aver. wgt. of liver (gm.) | 5.90 (5.2–6.3) | 6.45 (5.4–8.4) | 7.35 (7.1–7.5) | 6.30 (5.2–7.5) | 5.70 (5.2–6.4) | 5.50 (5.0–5.8) | 6.25 (4.9–7.9) |
| Aver. wgt. of spleen (mg.) | 865 (660–1,260) | 1,140 (719–1,948) | 541 (375–804) | 446 (337–536) | 233 (168–285) | 590 (431–664) | 452 (357–566) |
| Aver. wgt. of adrenals (mg.) | 50 (42–55) | | 61 (48–79) | 58 (51–72) | 79 (62–94) | | |
| Aver. wgt. of thymus (mg.) | 155 (110–206) | 280 (172–392) | 102 (73–139) | 79 (61–100) | 39 (33–61) | 94 (51–113) | 94 (65–126) |
| Aver. wgt. of cervical lymph node (mg.) | 73 (45–100) | 79 (54–117) | 55 (53–56) | 48 (34–70) | 23 (15–30) | 71 (54–90) | 59 (31–81) |
| Aver. wgt. of heart (mg.) | 584 (517–660) | 575 (512–648) | 584 (520–726) | 525 (451–587) | 585 (577–634) | 543 (483–626) | 463 (464–634) |
| Aver. wet wgt. of lungs (gm.) | 1.44 (1.25–1.71) | 1.45 (1.23–1.79) | 1.53 (1.07–2.39) | 1.09 (0.91–1.22) | 1.17 (1.01–1.34) | 1.45 (1.40–1.48) | 1.40 (1.32–1.51) |
| Aver. dry wgt. of lungs (mg.) | 306 (276–351) | 294 (259–346) | 343 (252–504) | 284 (223–331) | 314 (276–341) | 302 (295–308) | 305 (262–362) |
| Aver. per cent of water in lungs | 78.7 (77.9–79.3) | 79.7 (79.0–80.7) | 77.2 (75.2–78.9) | 73.8 (70.4–76.3) | 73.3 (72.7–73.9) | 79.1 (77.5–80.0) | 78.3 (76.1–79.4) |
| Aver. total white cell count | 12,260 | 13,000 | 3,240 | 450 | 300 | 1,600 | 1,070 |

The results are given in Table I and the several observations of interest are discussed under the appropriate group.

*Results*

*Group I.* The sham-operated control group on a restricted diet lost about 8 per cent of their body weight, representing a moderate degree of inanition. Necropsy findings were not remarkable. Organ weights are tabulated in Table I.

*Group II.* Two of the 10 adrenalectomized rats were sacrificed at 1 day, 2 at 2 days, and 6 at 8 days after operation; the organ weights of these animals, which were not appreciably different, are averaged together in Table I. The rats seemed well during the experiment. On 1 per cent saline drinking water, adrenalectomized animals gained 10 to 15 gm. in weight immediately after operation because of retention of fluid, which they then lost gradually. At sacrifice the thymuses and spleens were larger than those of Group II (controls, diet restricted). The lungs showed a slight increase in water. The other organs were not significantly altered, and no adrenal tissue was found. Leukocyte counts were normal, and the blood smears, while suggesting an increase in lymphocytes, were too few for valid interpretation.

*Group III.* Three mg. per kg. of $HN_2 \cdot HCl$ given subcutaneously produced severe effects in normal rats. At 24 hours the rats had lost a little weight and seemed moderately depressed, at 48 hours slight diarrhea appeared, and between 48 and 72 hours marked symptomatic changes developed. The rats appeared cold, were hunched-up, and huddled together. An almost continuous watery diarrhea, which contained considerable mucus, completely soaked the anal region and lower abdomen, and weight loss was marked. Neurologic changes were not noted, but the rats became more depressed and inactive. Those not sacrificed died between 80 and 102 hours.

Five rats were sacrificed at 24 hours, 4 at 48 hours, and 8 at 72 hours. The organ weights were averaged separately for each of these groups. At 24 hours the stomach contained a moderate amount of food, and in the small intestine small amounts of air and mucus were found. One animal had a small hemorrhagic patch in the ileum, and the mesenteric nodes were reddened. The thymus, spleen, and lymph nodes were decreased in size, and the adrenals appeared to be larger than is normal. The lungs were of normal weight but the fluid content appeared to be slightly reduced. The fall in white blood cell count was already marked and, although the lymphocytes were most severely depressed, the granulocytes had begun to fall also. At 48 hours the stomach was moderately filled with food, and the fluid in the small intestine appeared increased. The lymph nodes were hemorrhagic and those in the ileoce-

Aver. total white cell count
12,260
13,000
3,240
450
300
1,600
1,070

cal region were markedly so. The spleen and thymus were about half of the control weights and the lymph nodes were reduced in size. The suggestive increase in adrenal weight was still present. A remarkable finding was the sharp fall in the wet weight of the lungs, with retention of normal dry weight, indicating approximately 30 per cent decrease in the water content of the lungs. The average total leukocyte count had fallen to 450. At 72 hours, the stomach was distended with considerable food and fluid, and the intestines and cecum contained excess amounts of fluid, often including mucus. The colon was usually empty, but contained almost pure mucus in one animal. Small petechial hemorrhages often were found in the mesentery and hyperemic areas were present in the duodenum and lower ileum, the latter in one rat containing several deep erosions of the mucosa. Peyer's patches were hemorrhagic and the regional lymph nodes were small and red. The spleen, thymus, and lymph nodes were reduced to about 25 to 35 per cent of their control size. The increase in adrenal weight was unquestionable, and the livers were decreased in weight. The water content of the lungs remained decreased, being about 25 per cent of that of control group II.

*Group IV*. Rats receiving 3 mg. per kg. of $HN_2 \cdot HCl$ 24 hours after adrenalectomy showed a rapid acceleration in the development of toxic symptoms. Two animals died within 7 hours. At 24 hours the remaining rats appeared very weak, depressed, and had mild watery diarrhea; at 48 hours they were either dead or moribund; those remaining were huddled together, cold, moved with difficulty, and all died within 60 hours after injection. Diarrhea was moderate and weight loss was not marked. Four rats were sacrificed at 24, and 5 rats at 48 hours. At 24 hours the stomach was filled largely with fluid, the duodenum was slightly congested, and the small intestine greatly distended with clear fluid. The entire animal seemed excessively moist, and there was free peritoneal and thoracic fluid. The spleen, thymus, and lymph nodes were reduced in size, as markedly as those seen in the control animals treated with $HN_2 \cdot HCl$ (group III). The lymph nodes seemed slightly hemorrhagic. The water content of the lungs was slightly greater than is normal. The average total leukocyte count was 1,600. At 48 hours the rats were moribund when sacrificed. Their stomachs and small intestines were greatly distended with fluid. The walls of the duodenum were reddened and frequently hemorrhagic. The mesentery had a few small petechial hemorrhages and the fat had a peculiar white, granular appearance. The decrease in weight of the thymus and spleen was not much greater than that at 24 hours, and the weights of these organs did not differ significantly from those in group III. The water content of the lungs was only slightly decreased.

The average leukocyte count was 1,070 with 80 per cent lymphocytes; this total count was suggestively higher than that seen in group III.

*Microscopic Observations*

In the adrenalectomized rats injected with 3 mg. per kg. of $HN_2$·HCl, some intact lymphocytes could be found in the thymus, and generally the lymphatic tissue, although containing a diminished number of lymphocytes, appeared to be less severely affected than that of normal rats given the same dose. In another experiment, adrenalectomized rats, receiving 10 mg. per kg. of $HN_2$·HCl and sacrificed 3 to 6 hours after injection when they appeared moribund, showed severe lymphocytic fragmentation and chromatin débris in the thymus and lymph nodes, but again these effects seemed somewhat less than in the controls treated with the same dose. It should be noted that although 1 mg. per kg. of $HN_2$·HCl given subcutaneously, a sublethal dose in normal rats, is fatal to adrenalectomized rats, the histologic evidence of injury to the lymphoid tissue, bone marrow, and gastrointestinal tract is no more marked in the adrenalectomized rats than in the controls.

These experiments demonstrate that methyl-*bis* ($\beta$-chloroethyl) amine hydrochloride can induce lymphocytic destruction and involution of the thymus, spleen, and lymph nodes in the absence of the adrenal gland. Quantitatively, however, the lymphocytotoxic effect seems to be somewhat less severe in adrenalectomized rats than in normal ones injected with the same dose. The involution of the lymphatic tissue, therefore, results from a direct action of the nitrogen mustard, or is mediated by a different mechanism than that occurring in the "alarm reaction." Since, by definition,[5] the "alarm reaction" is involved in nitrogen mustard intoxication, it is possible that this mechanism may contribute to a slight extent to the lymphatic involution seen in rats intoxicated with $HN_2$·HCl. LeBlond and Segal[8] have been able to induce involution of the thymus in adrenalectomized rats by x-rays, although these effects were quantitatively less severe than those seen in normal rats. The nitrogen mustards seem to produce a somewhat similar effect.

DEMONSTRATION OF THE DIRECT ACTION OF $HN_2$·HCL INJECTED INTRAVENOUSLY, ON THE INTESTINAL TRACT *

It is shown in the following experiment that the intestinal injury induced by $HN_2$·HCl is due to a *direct* and rapidly completed action

* It had previously been shown in this laboratory that exclusion of the biliary secretion in rats did not prevent $HN_2$ from inducing characteristic intestinal injury. Since one could have anticipated this result from the experiment cited above, the biliary exclusion experiment is not described in detail.

of this compound on the intestinal tract. This demonstration was accomplished by temporarily occluding the circulation to the intestinal tract during varying, short periods of time after the intravenous injection of the compound, and then observing the nature of the injury produced.

Rats were anesthetized with ether, the abdomen opened, and most of the small intestine exteriorized and placed on cotton soaked with saline solution. Rubber-sheathed hemostats were placed about 5 cm. distal to the pylorus and 1 cm. proximal to the ileocecal valve, and a third hemostat was placed across the root of the mesentery. By this ring of hemostats, circulation to the major portion of the small intestine was occluded for 15 minutes. Control rats subjected to this procedure suffered no ill effects, and gave no evidence of significant histologic damage to the intestinal mucosa.

Following the application of the clamps, 2 mg. per kg. of $HN_2 \cdot HCl$ (1.6 $LD_{50}$) was injected into the vena cava, and 15 minutes later the clamps were removed. The rats were sacrificed at 60 and 72 hours. Grossly and microscopically the small portion of the duodenum and ileum outside the occluding clamps showed the characteristic injury due to the nitrogen mustards,[3] whereas the remainder of the small intestine, which had been surrounded by the clamps, was normal. The toxicity of $HN_2 \cdot HCl$, however, was not appreciably altered by this procedure.

This experiment clearly demonstrated that $HN_2 \cdot HCl$ had a rapidly completed, and presumably direct, action on the mucosa of the small intestine.

### DEMONSTRATION OF THE DIRECT ACTION OF $HN_2 \cdot HCl$ INJECTED INTRAVENOUSLY, ON THE BONE MARROW OF THE RAT AND RABBIT

It is shown in the following experiments that $HN_2 \cdot HCl$ has a direct and rapidly completed action on the bone marrow. This demonstration was accomplished by temporarily occluding the circulation to the hind legs during and for varying short periods after the intravenous injection of $HN_2 \cdot HCl$.

*Rats* were anesthetized with ether, the abdomen opened, and an arterial clamp placed on the abdominal aorta and inferior vena cava just below the renal vessels. Two mg. per kg. of $HN_2 \cdot HCl$ (1.6 $LD_{50}$) were then injected into the vena cava just above the clamp, and the clamp removed after intervals varying from 5 to 15 minutes. Operated controls carried the clamp for 15 minutes without ill effects and with no changes in the bone marrow. The rats were sacrificed 72 and 96 hours after injection.

At the time of sacrifice, the control rats injected with $HN_2 \cdot HCl$ had

extreme leukopenia, the leukocyte count being in the range of 50 to 250 per cmm. with cells usually too few to make a differential count significant. Microscopic sections showed the femoral, vertebral, sternal, and humeral marrow to be aplastic. The rats subjected to temporary vascular occlusion had leukocyte counts averaging 1700 per cmm. at 72 hours and 4,000 per cmm. at 96 hours after injection, with the differential count consisting predominantly of granulocytes. Histologic study (Figs. 1 and 2) showed aplastic sternal and humeral marrow, whereas the femoral marrow was extremely cellular and especially rich in myeloid elements. The marrow in the lumbar vertebrae below the level of the occluded aorta was very cellular, whereas that from the upper thoracic vertebrae was aplastic.

*Rabbits* were anesthetized with ether and, after suitable preparation of the skin, the aorta and vena cava were exposed through a left lateral abdominal incision. A rubber-sheathed hemostat was clamped on the two vessels during and for times varying for 2 to 15 minutes after the injection of 2 to 3 mg. per kg. of $HN_2 \cdot HCl$ (around the $LD_{50}$) into an ear vein.* Several complications may result from this procedure. If the clamp is left on too long (15 minutes or longer, usually), a gradual paralysis of the hind legs may occur, presumably due to injury in the spinal cord, since the legs do not become edematous, cold, or gangrenous. A dose of 3 mg. per kg. of $HN_2 \cdot HCl$ in the clamped animal frequently produces various neurologic manifestations such as tremors, ataxia, and convulsions, which in some instances seemed to lead to death within a period seen only when two to three times this dose is given to the normal rabbit. This would suggest, incidentally, that the lower part of the body accounts for a considerable amount of the chemical when it is injected into the intact animal. Because of these effects, a dose of 2 mg. per kg. with a clamping period of 2 to 5 minutes is recommended for a relatively uncomplicated preparation.

This experiment was repeated many times with uniform results in protecting the bone marrow of the lower part of the body.† The results obtained on 5 representative rabbits are detailed below, and the blood counts are shown in Text-Figure 1.

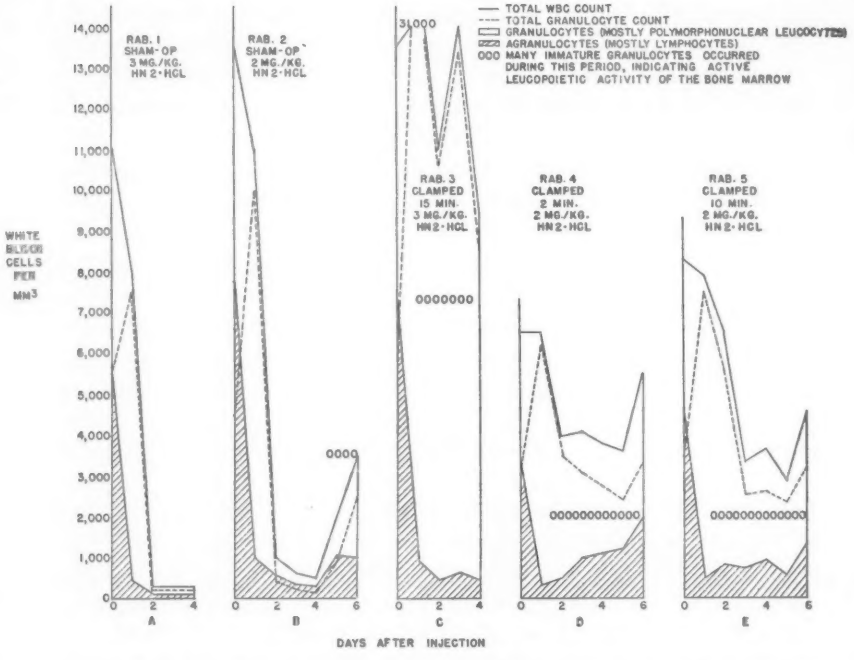
*Rabbit 1.* (*A Sham-operated Control.*) Rabbit 1 was given 3 mg. per kg. of $HN_2 \cdot HCl$ intravenously. Diarrhea appeared on the second day, weakness was progressive, and death occurred 92 hours after injection. The daily leukocyte and differential counts (Text-Fig. 1-A)

* It is possible to produce occlusion of the abdominal aorta and vena cava by inflating a blood pressure cuff around the rabbit's abdomen. The use of this simplified technic has not been thoroughly investigated as yet.

† Preliminary experiments have shown that the reticulocyte count in the clamped rabbit injected with $HN_2 \cdot HCl$ is severely depressed, although active regeneration of granulocytes is in progress. In view of the lymphocytotoxic action of the nitrogen mustards, this observation merits more detailed investigation.

were typical for the dose received. Typical pathologic findings were noted at autopsy, and there was no evidence of inflammation at the operative site.

*Rabbit 2.* (*Clamp Applied on Vena Cava for 5 Minutes Only, 2 mg. per kg. Injected.*) Rabbit 2 showed weight loss, diarrhea on the third day, and beginning recovery in weight 7 days after injection. The


TOTAL WBC COUNT
TOTAL GRANULOCYTE COUNT
GRANULOCYTES (MOSTLY POLYMORPHONUCLEAR LEUCOCYTES)
AGRANULOCYTES (MOSTLY LYMPHOCYTES)
MANY IMMATURE GRANULOCYTES OCCURRED DURING THIS PERIOD, INDICATING ACTIVE LEUCOPOIETIC ACTIVITY OF THE BONE MARROW
RAB. 1 SHAM-OP 3 MG./KG. HN2·HCL
RAB. 2 SHAM-OP 2 MG./KG. HN2·HCL
RAB. 3 CLAMPED 15 MIN. 3 MG./KG. HN2·HCL
RAB. 4 CLAMPED 2 MIN. 2 MG./KG. HN2·HCL
RAB. 5 CLAMPED 10 MIN. 2 MG./KG. HN2·HCL
WHITE BLOOD CELLS PER MM3
DAYS AFTER INJECTION
A
B
C
D
E

Text-Figure 1. Effects of the direct action of nitrogen mustard on the bone marrow of the rabbit. A and B illustrate leukotoxic action of nitrogen mustard at two levels of dosage. In C, D, and E the protective effect of clamping the aorta and vena cava for varying periods during which similar dosages of nitrogen mustard were injected is illustrated.

leukocyte and differential counts are shown in Text-Figure 1-B and are in accord with our previous observations for this dosage. Nine days after injection the rabbit appeared almost completely recovered.

*Rabbit 3.* (*Clamp Applied on Aorta and Vena Cava for 15 Minutes, 3 mg. per kg. Injected.*) The day following injection, rabbit 3 showed generalized neurologic symptoms. The animal was weak and incoordinate in its movements, and these symptoms were present until death. Three days after injection a very severe diarrhea appeared, and the rabbit became progressively weaker, dying 104 hours after injection. The leukocyte counts were unprecedented, as shown in

Text-Figure 1-C. The lymphocyte count fell precipitously and recovery did not occur. Granulocytes were greatly increased in number, and within 24 hours there was an increase in banded cells, polymorphonuclear leukocytes containing basophilic granulations, macropolycytes, and occasional myelocytes, this picture persisting until death. Ninety-six hours after injection the leukocyte count was 9,000 and the smear showed occasional myelocytes and normoblasts, and the differential count showed 11 per cent banded and 83 per cent segmented polynuclear leukocytes, 3 per cent basophils, and 3 per cent lymphocytes. At autopsy no gross inflammation was found; the incision appeared to be healing, and the intestines, as was expected, were distended with fluid. Smears of the bone marrow showed an aplastic humeral marrow and a hyperplastic femoral marrow.

*Rabbits 4 and 5. (Clamp Applied for 2 and 10 Minutes, Respectively, and 2 mg. per kg. Injected.)* Rabbits 4 and 5 developed the usual picture of $HN_2$ intoxication, except for the fall in granulocytes. Banded polynuclear leukocytes and pseudo-eosinophilic granulocytes with basophilic granulations were present 24 hours after injection and persisted during the period of observation. One animal survived, and the other died with purulent peritonitis 12 days after injection. The total leukocyte and differential counts are shown in Text-Figure 1, D and E.

*Comment*

These studies demonstrate that the action of $HN_2$·HCl on the cells of the bone marrow is a rapidly completed one, probably accomplished within less than 2 minutes after injection. This strongly suggests, therefore, that this compound has a direct action on the hematopoietic cells. It further shows that systemic intoxication does not interfere with the process of active granulopoiesis. Also, the data make clear that leukopenia, *per se,* is not essential in the lethal effects of the tested compound.

The availability of a technic whereby the major portion of the hematopoietic tissue of the body may be temporarily destroyed, while preserving a small area of actively regenerating bone marrow, should be of considerable use in other hematologic investigations.

## DEMONSTRATION OF THE DIRECT ACTION OF TRIS (β-CHLOROETHYL) AMINE HYDROCHLORIDE ($HN_3$·HCL),* INJECTED INTRAVENOUSLY, ON THE INTESTINAL TRACT

Clamping experiments on the intestinal tract, similar to those described above with $HN_2$·HCl, were carried out with $HN_3$·HCl in rats and rabbits. $HN_3$ produced effects similar to those reported for $HN_2$,

* $HN_3$·HCl is the official designation of the hydrochloride salt of this compound.

indicating that the former also has a rapidly completed action on the intestinal tract. It is probable that this will be true also in the case of the bone marrow.

### DEMONSTRATION OF THE DIRECT ACTION OF BIS (β-CHLOROETHYL) SULFIDE (H),* INJECTED INTRAVENOUSLY, ON THE INTESTINAL TRACT AND BONE MARROW

H is poorly soluble and unstable in water. Water, therefore, was not a suitable vehicle for intravenous injection, and solvents, such as propylene glycol and thiodiglycol, were used. This factor seems to have complicated a simple interpretation of the results obtained by the clamping technic.

#### *Methods and Results*

*Rats.* It was first shown that by occluding the circulation to the small intestine during and for 5 minutes after the intravenous (inferior vena cava) injection of 2.5 mg. per kg. of neat H, the clamped portion of the gut was protected from injury. Similarly, clamping of the abdominal aorta for 5 minutes during and for 5 minutes after the intravenous injection of 1 mg. per kg. of H in propylene glycol protected the bone marrow distal to the clamp, whereas the bone marrow proximal to the clamp was destroyed. These results were in accord with those obtained with the nitrogen mustards.

Needham, Cohen, and Barrett[9] reported, however, that they had been unable to obtain protection of the distal bone marrow in the rat against a dose of 2.0 mg. per kg. of H in thiodiglycol, injected intravenously, by applying a clamp to the abdominal aorta for 60 minutes. In order to resolve this discrepancy, 8 groups of 6 to 12 rats each were treated as shown in Table II. The results are summarized briefly therein.

This experiment confirmed both our previous experiment and the results obtained by Needham and co-workers.[9] The protective effect of vascular occlusion was found only when a dose of 1 mg. per kg. of mustard in propylene glycol was given. With a higher dose of mustard, or with thiodiglycol as a solvent, the protective effect of temporary vascular occlusion was not demonstrated. The explanation of the failure to obtain consistent protection of the distal bone marrow in these experiments is not apparent.

*Rabbits.* Seven rabbits were subjected to the clamping procedure, and then injected intravenously with 4.0 mg. per kg. of H in propylene glycol (1.5 $LD_{50}$). In 2 rabbits the mesenteric arterial supply to a 15 cm. segment of the ileum was occluded during and for 15 minutes

* H is the official designation of this compound.

after the injection, and one rabbit was sacrificed at 72 hours and the other at 96 hours. These animals both showed extremely severe injury to the spleen, thymus, and bone marrow with terminal leukocyte counts of 900 and 350 per cmm., respectively. The portion of the intestinal tract subjected to the vascular occlusion was practically completely protected, whereas the remaining portion of the small intestine showed severe damage.

TABLE II

*Protection Afforded the Femoral Bone Marrow of the Rat, by the Temporary Occlusion of Its Circulation, Against the Effects of the Intravenous Injection of H in Doses of 1 and 2 mg. per kg. in Propylene Glycol and Thiodiglycol*

| Group no. | No. of rats in group | Solvent used for injection | Intravenous dose of H | Duration of clamping | Effects on the bone marrow |
|---|---|---|---|---|---|
| | | | *mg./kg.* | *minutes* | |
| A | 6 | None | None | 5 | None |
| B | 6 | Propylene glycol | None | 5 | None |
| C | 12 | Propylene glycol | 1 | 5 | Severe leukopenia, but the femoral marrow was less severely injured than the sternal |
| D | 6 | Propylene glycol | 2 | 5 | Femoral and sternal marrow seem to be equally severely affected |
| E | 6 | None | None | 20 | None |
| F | 6 | Thiodiglycol | None | 20 | None |
| G | 6 | Thiodiglycol | 1 | 20 | Femoral and sternal marrow seem to be equally severely affected |
| H | 6 | Thiodiglycol | 2 | 20 | Femoral and sternal marrow seem to be equally severely affected |

Five rabbits had clamps applied to both the mesenteric artery and abdominal aorta during and for 15 minutes after the injection of H. One animal died soon after injection, and an autopsy was not performed in another dying 75 hours after treatment. Three of the 4 rabbits did not develop leukopenia, and at 72 and 96 hours, at the time of sacrifice, their leukocyte counts were 2250, 2850 and 4100 per cmm.; the remaining animal had a count of 600 per cmm. Histologic examination was performed on 3 rabbits. Again the temporarily clamped gut was protected, and the spleen and thymus in these animals were severely damaged. The rabbits all had extremely severe injury to the sternal, humeral, and upper vertebral bone marrow, whereas 2 rabbits showed complete and one rabbit (the one with a white count of 600 per cmm.) showed partial protection of the femoral bone marrow.

These experiments show, in general, what is much more clearly shown in the case of the nitrogen mustards, namely, that occluding the circulation to a given tissue before and for 15 minutes after the intravenous injection of mustard will serve to protect it from the action of the agent.

## DEMONSTRATION THAT THE BACTERIOSTATIC EFFECT OF RABBIT SERUM IS ALTERED BY THE INTRAVENOUS INJECTION OF $HN_2$ *

The demonstration that $HN_2 \cdot HCl$ has a rapidly completed action and disappears from the blood shortly after injection makes it necessary to find a secondary mechanism which is more directly responsible for the development of systemic intoxication. A study of alterations in the circulating blood of intoxicated animals was undertaken, and the results have been reported elsewhere in this series.[10] Among various approaches, the measurement of bacterial growth in serum as a possible method of demonstrating the appearance of toxic substances in the serum was considered. A simple experiment was performed, and this is briefly reported because of the unexpected results obtained.

### *Methods and Results*

The growth of type A hemolytic streptococcus ($C_{203}$) was determined in normal rabbit serum and in serum taken at various intervals after the intravenous injection of 3 mg. per kg. of $HN_2 \cdot HCl$. Blood was obtained by cardiac puncture, centrifuged, and the serum removed and refrigerated. One-tenth cc. of a $10^{-2}$ dilution of an 18-hour culture of washed streptococci was implanted in 0.9 cc. of each sample of serum, and colony counts were made at 0, 4, 8, and 24 hours after incubation.

The control serum of 4 rabbits, whether starved or fed, was bacteriocidal, bacteriostatic, or permitted slight growth of bacteria over the period of 8 hours' incubation. In these same rabbits, 6 samples of serum drawn within 6 hours after injection of $HN_2 \cdot HCl$ showed very little difference from the controls. Following this 6-hour period, and until the rabbits died, 3 to 5 days later, the serum became much more favorable for the growth of bacteria, and the rate of bacterial growth was strikingly accelerated.

Although the variability of bacterial growth in normal rabbit serum prevents any quantitative statement, enhanced bacterial growth of hemolytic streptococci occurred consistently in the serum of the intoxicated animals beginning 6 to 8 hours after the injection of an $LD_{50}$

* We wish to thank Dr. Colin MacLeod and Mrs. Edna Stone of the Department of Bacteriology, New York University College of Medicine, for assistance in carrying out this study.

dose of $HN_2 \cdot HCl$. This observation suggests that some alteration occurs in the blood beginning at 6 to 8 hours after injection which facilitates the growth of the test bacteria. It was not possible to pursue this observation further.

## Summary and Conclusions

1. Methyl-*bis* ($\beta$-chloroethyl) amine hydrochloride ($HN_2 \cdot HCl$) was used as the type substance in this study. *Tris* ($\beta$-chloroethyl) amine hydrochloride ($HN_3 \cdot HCl$) and *bis* ($\beta$-chloroethyl) sulfide (H) were examined in more limited trials and, in the particulars tested, gave essentially the same results as did $HN_2 \cdot HCl$.

2. The "alarm reaction" of Selye must be considered in interpreting the toxic effects of any drug. It is shown that only a minor and questionable rôle can be ascribed to this reaction in the production of the characteristic pattern of injury to the lymphatic and hematopoietic tissues and intestinal mucosa resulting from $HN_2 \cdot HCl$ intoxication. Following the injection of $HN_2 \cdot HCl$ in the rat, involution of the lymphatic tissue and lymphocytic destruction occur in the absence of the adrenal glands.

3. That the damage to the intestinal tract and bone marrow is a rapidly completed and presumably direct action of the agent, and is not related to the "alarm reaction," was shown by the following procedures:

a. By means of occluding the circulation to a portion of the small intestine during and for 5 to 15 minutes after the injection of a lethal dose of $HN_2 \cdot HCl$, that portion was protected from the damaging effect of the compound.

b. By means of occluding the circulation to the lower extremities by a clamp on the abdominal aorta and inferior vena cava during and for 2 to 15 minutes after the intravenous injection of $HN_2 \cdot HCl$, granulocytopenia in the peripheral blood was prevented, and at autopsy hyperplasia of the femoral marrow, with aplasia of the rest of the bone marrow in the body, was observed.

4. Beginning 6 hours after the injection of a lethal dose of $HN_2 \cdot HCl$ in the rabbit, the growth of hemolytic streptococci in the serum is enhanced. This suggests that an alteration occurs in the blood making it more favorable for the growth of bacteria.

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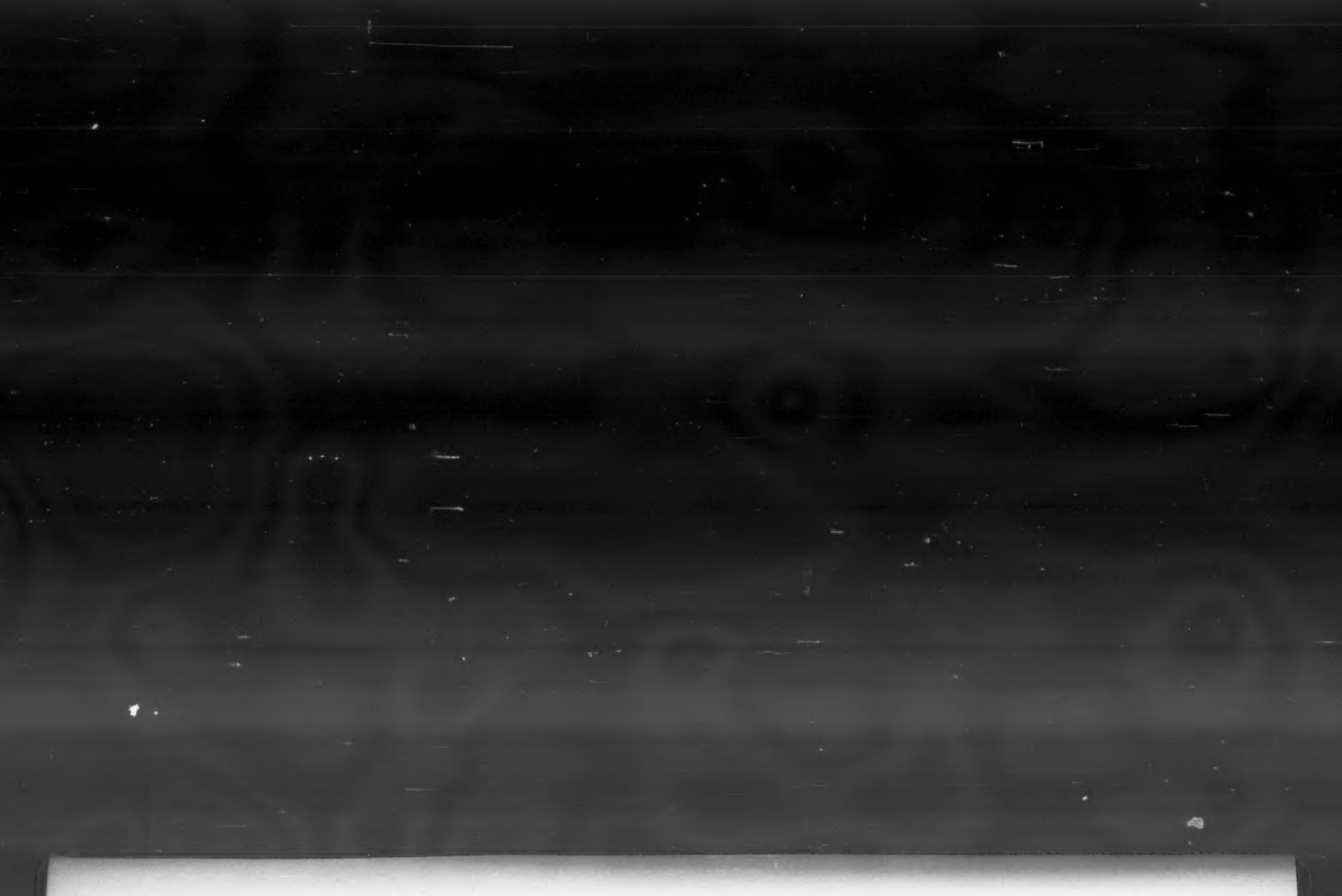
## DESCRIPTION OF PLATE

### PLATE 57

FIG. 1. Photomicrograph of a section of the sternum of a rat given 3.0 mg. per kg. of $HN_2 \cdot HCl$ intravenously and sacrificed 70 hours afterward. The sinusoids are more prominent due to engorgement with red blood cells. The marrow is represented by fat spaces and cells and reduced numbers of stem cells imbedded in a matrix containing protein precipitate. Eosin and azure II stain.

FIG. 2. Femoral bone marrow of a rat protected from intoxication by $HN_2 \cdot HCl$ by clamping the aorta for 10 minutes during and after the intravenous administration of 2 mg. per kg. Eosin and azure II stain.



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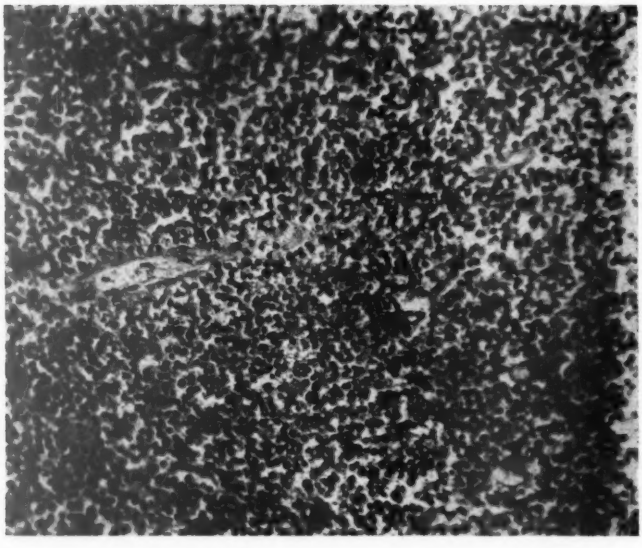

Karnofsky, Graef, and Smith Effects of Nitrogen and Sulfur Mustards

# HISTOLOGIC STUDIES ON A VIRILIZING TUMOR OF THE ADRENAL CORTEX *

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New growths of the adrenal cortex are frequently associated with an increased excretion of 17-ketosteroid hormones. Steroid hormones are formed normally in the cortical cells through a series of chemical transformations which parallel cytoplasmic changes, and are elaborated in excess by tumors. The neoplasms arise in different age periods, namely, fetal, juvenile, and adult. The fetal tumors arise in females and produce pseudohermaphrodism. The juvenile tumors have their origin in children of either sex and are associated with virilism and the precocious development of secondary male characteristics. Tumors of the adult give rise to heterosexual changes. The studies to be described deal with the histologic changes found in an adrenal cortical tumor, removed surgically, from a 4-year-old male, who showed advanced secondary sex characteristics and an increased urinary excretion of 17-ketosteroid hormones.

Reports of 21 authentic cases of virilizing adrenal cortical tumors occurring in males between the ages of 11 months and 16 years have been found in the literature.[1-21] Eight other cases have been recorded,[22-25] but the data were insufficient to justify their inclusion among the proved cases. The tumor was removed surgically from 9 patients [4, 5, 12, 18, 21] with 2 survivals,[16, 18] and hormone assays were reported from 5 patients.[3, 5, 12, 18, 21] Seventeen of the records included brief histologic descriptions except in the report of Goormaghtigh.[6] In a study of the cellular characteristics of adrenal cortical neoplasms, Goormaghtigh described in 2, virilizing tumor cells showing transformation of mitochondria into granules which gradually were converted into clear droplet-like structures. These droplets, after the discharge of their contents into the neighboring lymph channels, remained as vacuoles. He had previously published a study on the chronologic cellular development of the adrenal cortex and later attempted to correlate the cytologic features of the normal tissue with those found in different adrenal cortical tumors.[26]

## CLINICAL HISTORY

P. M. P., a white male, 3½ years of age, was admitted to Children's Hospital on March 9, 1945, as a patient of Dr. J. W. Leech. The patient had had a gradually enlarging abdomen since birth and a generalized growth of fine hair, but the

* Received for publication, April 30, 1947.

latter had not attracted particular attention. He was the first of two children born to apparently normal parents, and no similar conditions were known in any member of either family. The past medical history was not pertinent. Physical examination revealed a well developed musculature. In the right upper quadrant of the abdomen, a large mass was felt which extended 5 cm. below the costal margin in the mid-clavicular line. This mass showed, roentgenographically, some fine areas of calcification and was tentatively diagnosed as liver. Other physical findings, except a moderate tachycardia, were normal. After 1 week, the patient was discharged to be observed at home. In May the parents consulted Dr. Joseph Stokes, Jr., of Philadelphia who, on the basis of pyelographic examinations, diagnosed the mass as a tumor of the right adrenal.

The patient was readmitted on October 2, 1945. At that time a definite increase in the growth of the beard and the pubic and axillary hair was noted. The genitalia resembled those of a 16-year-old male. Acne had developed over the face and neck. The blood pressure was 190/160 mm. Hg. The right ocular fundus was swollen and hemorrhagic with an obscured optic disk. The urine and blood cellular pictures were normal. The bone age was approximately 4 years. On October 5, an exploratory laparotomy was performed by Dr. J. W. Shirer. The liver was displaced downward and forward by a large, nodular, encapsulated mass in the region of the right adrenal. This mass was separate from the kidney and was not attached to the diaphragm. No evidence of metastasis was seen in the liver, and the left adrenal appeared normal in size and shape. On October 5, a steroid assay by a modified Zimmermann[27] method on a 24-hour urine specimen gave a value of 14.7 mg. of 17-ketosteroid. On October 15, the tumor was removed by Dr. Shirer through a flank incision, and the patient made a rapid recovery with no evidence of adrenal cortical insufficiency. After operation, the blood pressure fell to 90/60 mm. Hg and has remained in this range. On October 17, a 24-hour urine specimen gave 5.4 mg. of sodium pregnandiol glucuronidate on an analysis by the Venning method.[28] There was no precipitable free steroid. The patient was discharged from the hospital on October 28, 1945.

On November 14, 1945, the 17-ketosteroid level was 4.8 mg. with a 24-hour output of 600 cc. of urine, and on January 2, 1946, a similar low level of 4.7 mg. was obtained on a 24-hour output of 274 cc. of urine.* The normal urinary 17-ketosteroid level for a 3½-year-old male is 5 mg. for 24 hours and for an adult male, 30 mg. per 24 hours.[29,30]

### *Pathologic Data*

The tumor, which included the right adrenal, was an encapsulated, nodular mass, measuring 10 by 10 by 7 cm. The blood supply entered through a pedicle. The cut surface showed two types of tissue: one, which comprised most of the tumor, appeared yellow and fatty with many small stellate areas of calcification; and a second, which consisted of small peripherally located areas, was reddish and relatively soft and friable. The tumor was preserved in Jores' solutions, and blocks were fixed in Zenker's solution, and in equal parts of 95 per cent alcohol and 10 per cent formalin. The stains used on fixed tissue were hematoxylin and eosin, Masson's trichrome, ponceau fuchsin and aniline blue, iron hematoxylin, and van Gieson's. Frozen sections were stained with sudan IV, osmic acid, and ponceau fuchsin.

* We are indebted to Dr. H. S. Strickler, Department of Biochemistry, University of Pittsburgh School of Medicine, for these analyses.

Microscopically, the tumor was made up of an anastomosing network of tissue enclosing vascular channels, architecturally similar to the zona reticularis of the normal adrenal cortex. The strands of the network were composed of two main types of tissue; one predominantly cellular (Fig. 1), and the other hyalin-like and relatively acellular (Fig. 2). Various intermediary stages were found between these two types (Fig. 3). The cellular portion ranged from thick double-celled strands of polygonal cells (Fig. 1) through gradually narrowing cords (Figs. 3 and 4) to small, shrunken, degenerated cells with pyknotic nuclei (Figs. 2 and 3). The degenerated forms were the end result of progressive secretory changes. In the normal gland, the secretory phases, which have been designated pre-secretory, secretory, post-secretory, and senescent, occur chronologically and are aligned with definite cytologic characteristics. In the tumor studied, comparable cytologic patterns, with the exception of the earliest cell type, have been established. Eight different cell types were demonstrated. Six of the eight types occurred in the cellular portion, and the remaining two were senescent cells associated with the hyaline cords. The cells and nuclei of the cellular tissue varied in size depending upon the phase of activity. The largest cells occurred in the secretory phase. The earliest stages of the pre-secretory phase were difficult to find, and the parent cells, which originate in the capsule of the normal adrenal, could not be identified with certainty. What we believe to be the immediate successors of the parent cell were found, but in small numbers only. This earliest identified cell type belonged in the pre-secretory phase and averaged 10 $\mu$ in diameter. The nuclei, which were occasionally paired, were relatively large, and the cytoplasm was compact, homogeneous, and stained diffusely with eosin (Fig. 6a). The next development was the formation throughout the cytoplasm of dust-like indistinct granules which gave the cytoplasm a finely granular, pink appearance when stained with ponceau fuchsin (Fig. 6b). These granules form the initial stage in the genesis of the mitochondria. This pre-mitochondrial material gradually became segregated as larger, irregular granules staining black with iron hematoxylin and red with ponceau fuchsin (Fig. 6c). These granules, which apparently arose through cohesion of the denser components of the cytoplasm, gradually became more compact and were surrounded by a clear spherical zone (Fig. 6d). The four successive cell changes ending with the establishment of the discrete granules constituted the pre-secretory phase.

Gradually each distinct, small mitochondrial mass was transformed into a large globule, staining red with sudan IV or ponceau fuchsin. This globular formation constituted the secretory phase. The cells of

the secretory phase reached approximately 25 $\mu$ in diameter, and this increase in size apparently was due to expanding secretory globules. The largest globules were found adjacent to the cell wall bordering the vascular channel, and the smaller ones deeper in the cytoplasm (Fig. 7a, b, c). The globules were bounded by a more deeply staining, thin peripheral layer. When observed with polarized light, some of the globules contained, or were intimately associated with, doubly refractile material, obviously cholesterol. In the frozen sections, the globules stained magenta red with ponceau fuchsin and orange with sudan IV. With routine stains, the globules were not discernible, although in the denser areas of the cytoplasm diffuse, reddish-staining, finely granular material was observed. The contents of the globules were readily soluble in fat solvents and were not retained within the cell after prolonged fixation (Fig. 5) or after treatment with xylol. When xylol was applied to frozen sections stained with ponceau fuchsin, the outward diffusion of the reddish contents could be seen readily. In some of the frozen sections made from tissue fixed for a relatively short time and stained with ponceau fuchsin, masses of homogeneous red material, which had either been secreted *in vivo* or had diffused from the contiguously lying cells during fixation, were seen in the vascular channels. The latter process seemed to be the more probable. Frozen sections made at successive intervals during prolonged fixation showed a diminishing capacity of the globular contents to stain. The staining capacity was completely lost in tissue after fixation for 4 months and the secretory cells contained mainly empty vacuoles (Fig. 5). With the *in vivo* diffusion of the contents of the secretory globule into the blood stream, the secretory phase was ended. The post-secretory cell was smaller than that of the previous phase, and cells could sometimes be found in which the cytoplasm contained empty vacuoles with definite boundaries (Fig. 8).

The senescent phase was characterized either by swelling or shrinking. When swelling occurred, the nuclei became enlarged and karyolytic, following which the cells appeared fragmented and disappeared (Fig. 9a). When the nuclei became pyknotic, there was a diminished cytoplasm in which fibrils occasionally appeared (Fig. 9b). These fibrils stained blue with Masson's stain or red with eosin and apparently arose from the dark-staining walls of the post-secretory vacuoles. These fibrils varied in number and thickness, and were associated with the developing hyalin. In some of the degenerating cells stained by Masson's method, transformation of the cytoplasm into hyalin could be followed by variations in color (Fig. 10). The normal reddish-colored cytoplasm became bluish in patches in which the fibrils were embedded.

These blue patches extended and finally filled the entire cell. With the conversion of the cytoplasm into hyalin, remnants of the finally depleted senescent cells were seen either as a persistent thin network of pyknotic nuclei with a minimal amount of cytoplasm bordering the vascular channel, or as a conglomeration of cast-off nuclei lying within the vessel. These desquamated cells were very evident in sections stained with iron hematoxylin. It was difficult to establish whether the fibrils arose from the walls of the empty vacuoles or whether the degenerating cytoplasm contributed to their formation. The cellular changes following secretion were rapid and difficult to follow with certainty. The amount of hyalin was in inverse proportion to the quantity of residual cytoplasm in the degenerating cellular elements.

## Discussion

In the adrenal cortex, an orderly progression of cells from the capsular region through the glomerular and fascicular zones to the zona reticularis has been recognized since 1883.[31] Studies concerned with this progression were reported by Hoerr,[32] Bachmann,[33] and Wotton and Zwemer,[34] all of whom indicated that the primary cell migrated through the cortex and during its migration underwent metabolic and morphologic changes with final eradication. Goormaghtigh,[6] Bennett,[35] and Swinyard [36] have added the minute cytoplasmic changes which occur in chronologic order throughout the progression. The cytoplasmic transformations are associated with the chemical formation of a progressive series of steroid hormones. There is little definite information available at present concerning this series, the development of which is under investigation by various workers. The cellular changes, which have been followed in the tumor studied, roughly duplicate those in the normal cortex; however, changes in the normal cortex occur in an orderly progression, divisible into anatomic zones, but those in the tumor, although they are represented by similar cell types, do not occur in an orderly architectural fashion.

Our most interesting observations deal with the fatty secretory globules, which stain with ponceau fuchsin and are identical with those described by Broster and Vines [3,37] with this stain. These authors believed that the positive staining reaction was a definite indication of the presence of a male steroid hormone, a postulate which has not been substantiated by other workers to date. The material contained in the globules is readily soluble in fat solvents and is dissolved from the cell in any technic requiring paraffin embedding. The only procedures which gave positive globular staining in our hands were those carried out on frozen sections. Such material staining with ponceau

fuchsin is not only readily soluble in xylol but also soluble, although in less degree, in aqueous solution. It is interesting that frozen sections, made from the tumor material shortly after placing in Jores' solution II, contained globules filled with deeply-staining material. After some weeks of immersion in Jores' solution there was a marked reduction in stainable material in the cells. The rapid outward diffusion of the stained contents of the globules when placed in suitable solvents demonstrated the lability of the contents and explained the formation of the vacuoles. The presence of vacuoles with a lack of stainable content in the carcinoma of the adrenal cortex studied by Goormaghtigh [26] is probably explained by the diffusion of the globular content during preservation and fixation. Attempts by us at histochemical demonstration of androsterone *in situ* were not successful because, first, the amounts in the tissue were decreased by diffusion, and, second, the reagents used drastically altered the tissue. Proof of the elaboration of male sex hormones by the tumor is indicated by the functional and anatomic changes induced by the tumor and their disappearance on removal of the new growth.

The recent work of Mason and Kepler [38] demonstrates that in a majority of the virilizing tumors of the adrenal cortex, the predominating urinary hormone is dehydro-iso-androsterone with a small amount of androsterone. The presence of dehydro-iso-androsterone in large amounts in urine is practically diagnostic of virilizing adrenal cortical tumors. The association of cholesterol with the material staining with ponceau fuchsin suggests cholesterol as a basic substance, which by oxidation gives rise in the adrenal cortex to a series of adrenal steroids numbering at least 30.[39]

## Summary

In a virilizing adrenal cortical tumor removed from a boy, 3½ years old, a progressive series of cells were identified which paralleled in morphologic sequence and associated secretory changes a comparable series of cells occurring in the normal gland. The secretion, which was associated with the formation of hormones, could be demonstrated histologically with ponceau fuchsin and sudan IV and was removed from the cell by fat solvents.

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## DESCRIPTION OF PLATES

### PLATE 58

FIG. 1. Islands of secretory cells surrounded by endothelium-lined sinuses which contain red blood cells. × 700.

FIG. 2. Hyaline cords containing remnants of a few degenerating nuclei and many extruded senescent cells in different stages of degeneration bordering the sinuses. × 700.

FIG. 3. Gradual degradation of secretory cells into hyalin. Cells in the secretory phase are shown in the upper third of the section. In the central part the cells are in the process of post-secretory degeneration. The cells in the lower third are undergoing hyalinization. × 225.

FIG. 4. Early stage in the degeneration of secretory cells. × 700.

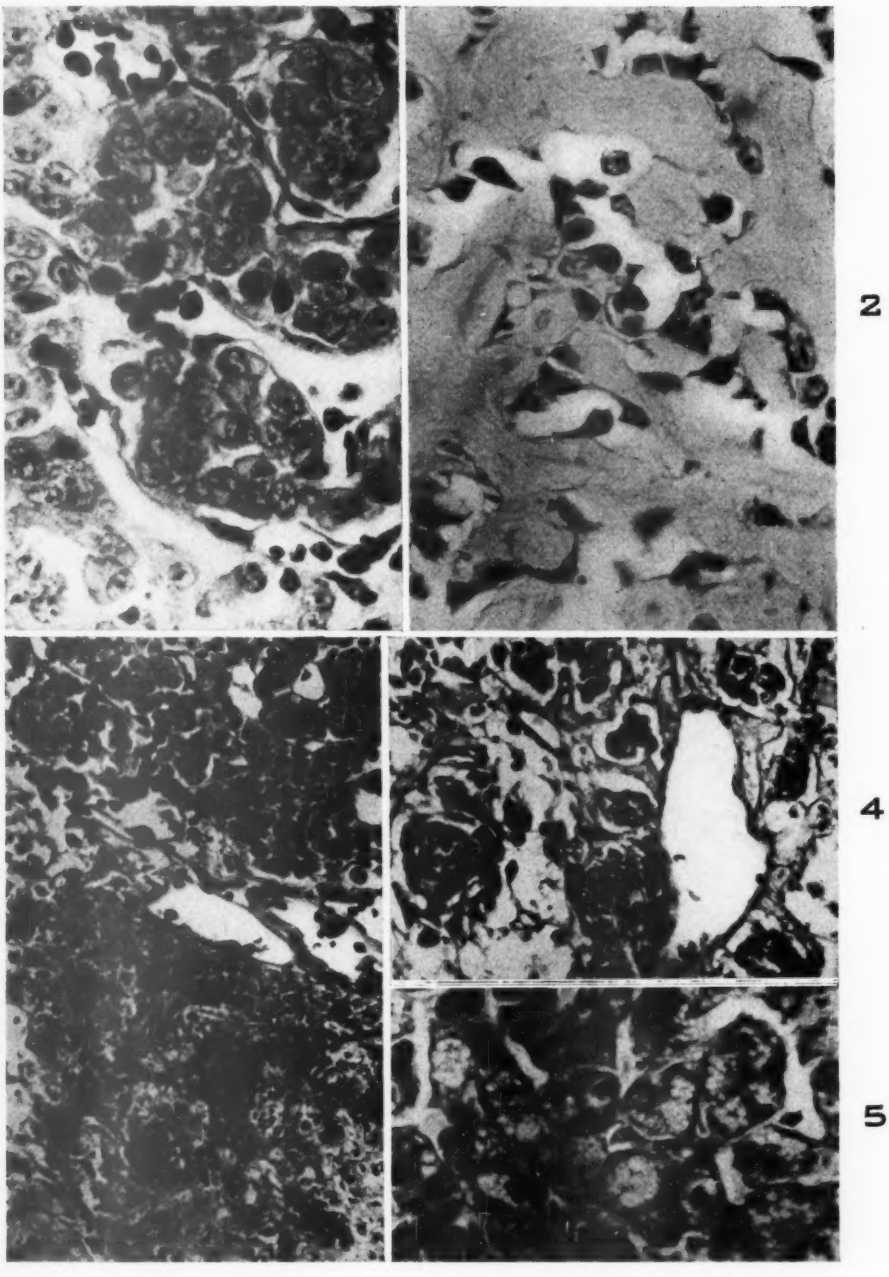
FIG. 5. Vacuoles in tissue from which the secretory contents have diffused during a fixation period of 4 months. × 225.

A

1

3

W




Weber and Menten Virilizing Tumor of Adrenal Cortex

## PLATE 59

FIG. 6a, b, c, d. Photographs of camera lucida drawings showing the progressive development of granules in the pre-secretory phase. × 1800.

FIG. 7a, b, c. Photographs of camera lucida drawings of cells in the secretory phase showing (a) secretory globules in cytoplasm bordering vascular channel, (b) globules immediately before expulsion of contents, (c) extrusion of globular contents into sinus. Globules stain magenta red with ponceau fuchsin stain. × 1800.

FIG. 8. Photograph of camera lucida drawing showing vacuoles in a post-secretory cell after contents have been secreted into a blood sinus. × 1800.

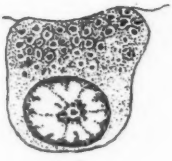
FIG. 9a, b. Photograph of camera lucida drawing of senescent cells showing (a) fragmentation of cytoplasm and karyolysis of nucleus, (b) shrinking of cytoplasm and pyknosis of nucleus. × 1800.

FIG. 10. Photograph of camera lucida drawing showing the formation of hyalin in the cytoplasm of two senescent cells bordering a sinus. The homogeneous cytoplasm (stained red in section) is being converted into fibrils (stained blue in section). Masson's stain. × 1800.

6a

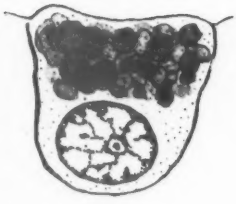
6b

6c



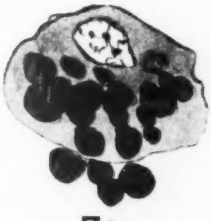
6d

10

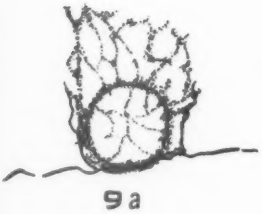


7a

8

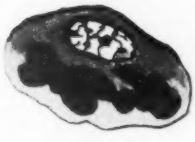


7c



9a

9b



7b

Weber and Menten

Virilizing Tumor of Adrenal Cortex

# TUMORS OF THE CAROTID BODY*

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Tumors arising in the carotid body or glomus caroticum have been called by many names, including perithelioma, endothelioma, angioma, chromaffinoma, adenoma, paraganglioma, pheochromocytoma, and sympathoblastoma. The variety of names reflects the current confusion regarding the development, structure, and function of the carotid body. Accordingly, a consideration of some of the contemporary ideas on these subjects is in order.

## *Embryology*

The carotid body was regarded by Kohn [25-27] as being derived from cells of the sympathetic nervous system and from the same anlage as the sympathetic ganglia. This concept has dominated the field until recent times, when considerable doubt has been cast upon it.

Smith [42] regarded the carotid body as a complex of elements which become associated during the developmental history of the third mesodermal arch. She indicated that mesodermal cells and neural elements from the glossopharyngeal, vagus, and sympathetic nerves might all participate. Boyd [4] emphasized a mesodermal condensation related to the third branchial arch artery and indicated his belief that these cells persist to form a considerable portion of the essential cells of the adult structure. He acknowledged also contributions from glossopharyngeal, vagus, and sympathetic nerves, but felt that these were of secondary importance.

The possibility that a few sympathetic cells are included in these bodies cannot be denied, especially since, as noted by Hollinshead,[19] the difficulties of following wandering embryonic cells in the region of the branchial arches are considerable. It seems probable, however, that even if migratory elements from the sympathetic ganglia are included in the carotid body, their rôle is a minor one.[4, 19, 42]

## *Anatomy*

Grossly, the carotid body is a soft, but tough, ovoid, pale tan, rather poorly defined mass measuring about 5 by 3 by 2 mm. It is situated usually a little medial to and behind the bifurcation of the common

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carotid artery, being rather firmly bound to the vessels by loose fibrous tissue.

Microscopically, the tissue may be arranged in more or less distinct lobules (*e.g.*, cow, man) or in a compact mass (cat). The essential or "specific" cells are arranged in whorls or clusters (called "Zellballen" by Kohn [25]) which are completely surrounded by a supporting stroma exceedingly rich in capillaries (Figs. 1 and 2). This fundamental and characteristic structure is brought out clearly by silver impregnation of the reticulum (Fig. 3). The cells making up these clusters are round or polyhedral, resembling epithelium, with round or slightly oval nuclei containing fine chromatin particles. Their cytoplasm is abundant and may appear granular, vacuolated or reticular, depending in part upon fixation and technic. Cell boundaries are usually distinct, and there appears to be no good evidence that they ever form a syncytium. The presence of ganglion cells [15,33] has been seriously questioned by Hollinshead.[21]

The supporting stroma consists of fine collagenous and reticular fibers with innumerable capillaries. The endothelial cells of the latter are so large and numerous that some may appear to lie outside of the capillary lining (the so-called "perithelial" cells). Some of them are perhaps homologous with the "neuro-myo-arterial" cells of the cutaneous glomus.

In specially prepared sections, numerous nerve fibers are found. Thus, the principal constituent elements of the carotid body may be considered to be the "chief" or epithelioid cells making up the cell clusters, and the neural and endothelial elements (the latter perhaps including "perithelial" cells).

### *The Chromaffin Reaction*

Much of the confusion regarding the nature of the carotid body and the histogenesis of its tumors arises from the use of the so-called chromaffin reaction. This refers to a yellowish brown color assumed by certain cells after fixation in fluids containing chromium salts. The prototype of this reaction is seen in the cells of the adrenal medulla, where the material taking the brown color is regarded as a precursor of epinephrine. Other cells giving the reaction are found in association with sympathetic ganglia, particularly in the abdomen.

Kohn [25] laid particular emphasis on the presence of the chromaffin reaction in some cells of the carotid body in various animals and regarded this as important evidence for his assumption of a sympathetic origin for the specific cells. Cells not giving the chromaffin reaction were regarded as of sympathetic origin nonetheless. Smith [42] and Boyd [4] found the chromaffin reaction to be present in a portion of the

cells of the carotid body in certain species (*e.g.*, cow, pig), but absent in others (*e.g.*, rat, man). DeCastro,[7,8] in a careful study, questioned the existence of a true chromaffin reaction in the carotid body and stated that the apparent reaction was due to the presence of lipoid substances in the cells. This conclusion was largely confirmed by Hollinshead,[21] who, however, felt that the cytoplasmic granules were not lipoid in nature, but might be mitochondria.

The chromaffin reaction has been clearly shown to be nonspecific. Indeed, the word "chromaffin" is a misnomer, since various oxidizing agents other than chromium salts will give the reaction.[12] Furthermore, organic substances other than epinephrine will form brown compounds when oxidized, *e.g.*, hydroquinone, resorcinol, aniline, various aldehydes and ketones, polyphenols, and polyamines.[1]

### *Innervation*

The innervation of the adrenal medulla, with which Kohn [25] supposed the carotid body to be homologous, is motor and predominantly preganglionic. The carotid body, on the other hand, receives chiefly sensory nerves from the glossopharyngeal nerve.[7]

### *Function*

The carotid body is sometimes referred to as a gland of internal secretion [11] or as having no known function.[43] The suggestion of DeCastro [8] that it is a chemoreceptor apparently has received abundant confirmation in the work of Heymans and Bouckaert,[18] of Schmidt and Comroe,[41] and of Dripps and Comroe,[9] who have shown that it and the similar cardio-aortic bodies are sensitive to changes in the pH, and in the carbon dioxide and oxygen tensions of the circulating blood, and that under certain conditions they may be of major importance in the regulation of respiration. It is also noteworthy that extracts of the carotid body have not been shown to contain epinephrine.[5]

### *Classification of the Carotid Body*

Kohn's classification of the carotid body with the "paraganglia" [25-27] has had great influence down to the present day. Apparently he used the term to apply to collections of tissue associated developmentally with the sympathetic ganglia and homologous with the adrenal medulla. As noted by Hollinshead,[19] if this classification is to be acceptable for the carotid body, the latter should be shown to be similar to the adrenal medulla in development, structure, innervation, and function. However, as outlined above, the two structures appear to differ on all these counts. Similar observations apply to the aortic bodies or cardio-aortic bodies,[3,36,37] and it seems extremely doubtful that either they or the

carotid body should be included with the paraganglia. The propriety of regarding the carotid body as an arteriovenous anastomosis [15] or "glomus" is also questionable, since Hollinshead [20] has shown that it differs in vascular arrangement, innervation, and cytologic features from the glomus coccygeum, which presumably does represent a specialized arteriovenous anastomosis. It appears, therefore, that the carotid body, together with the similar cardio-aortic bodies, should be classified as a specialized chemoreceptor and not as a gland of internal secretion, paraganglion, or arteriovenous glomus.

## Tumors

The present report is based upon 17 tumors taken from the files of the New England Deaconess Hospital and the Laboratory of Pathology of the Harvard Cancer Commission.* Without exception, they were removed surgically from the region of the bifurcation of the common carotid artery. In one case, only tissue taken for biopsy was available.

### *Gross Appearance*

Weights were not recorded for most of the tumors. Three weighed 28, 50, and 60 gm., respectively. Measurements varied from 3 by 3 by 2 to 5 by 4 by 3 cm. In most cases the shape was globular or ovoid, a groove for the carotid artery sometimes being present if the artery had not actually been resected. In cases in which it was necessary to remove a segment of the artery, the neoplastic tissue was found to be closely adherent to the wall of the vessel. A well defined capsule usually was present. The color was recorded as pinkish gray to reddish brown. The tissue was usually firm and homogeneous.

### *Microscopic Features*

Microscopically, most of the tumors showed a tendency to mimic the structure of the normal carotid body. The usual pattern was one of nests of the "chief" or epithelioid cells, fairly uniform in size, and surrounded by a vascular stroma similar to that of the normal organ. There was usually more variation in size and shape of the cells than in the normal gland. Mitotic figures were not observed. Generally speaking, the cell clusters of the tumors were larger than those of the normal organ (Figs. 4 and 5), and the supporting stroma was less cellular. The general pattern is best brought out by silver impregnation of the reticulum (Figs. 6 and 9).

For descriptive purposes, it was found possible to subdivide the tumors into three groups. The first, or *usual* type, included most of

* The clinical aspects of 15 of these cases have been reported separately by Lahey and Warren.[30]

the cases (12 tumors) and is the variety in which there is more or less faithful reproduction of the normal structure (Figs. 4, 5, 7, and 8). The second, or *adenoma-like* type (2 tumors), shows a pattern in which the chief cells have a pronounced epithelial appearance, with rounded or polyhedral shape, abundant cytoplasm, and arrangement in sheets or rows (Figs. 10 and 11). This appears to be the type referred to as adenoma by some authors.[11] In this type the reticulum is scanty. The third type may be called *angioma-like* (2 tumors). Here the cells are largely spindle-shaped or crescentic and apparently closely related to capillaries (Figs. 12 and 13).

In spite of these variations, however, the fundamental pattern appeared to be the same. The structural unit consisted of a group, of variable size, of the chief or epithelioid cells, surrounded by a more or less abundant vascular stroma. Most of the variations in microscopic appearance of the tumors seemed to be produced by differences in the shape of the chief cells. This in turn was apparently influenced to no small degree by such factors as clamping and squeezing the tissue during surgical removal, the time elapsing between removal and fixation, and the type of fixative. In many instances, more or less flattened or spindle-shaped cells were found in part of a tumor, while in better preserved fields the cells were plump and rounded. The mechanical factors involved in the growth of a well encapsulated tumor in an environment naturally restricted by dense fascial planes may also be considered as influencing the shape of the cells.

Most striking was the persistence of the general pattern of the reticulum in the majority of cases (Figs. 6, 9, and 13). Although the number of fibers and the size of the cell groups which they surrounded varied, the basic arrangement remained the same.

### *The Chromaffin Reaction*

The cytoplasm of the chief cells was, in varying degrees, granular, vacuolated, or reticulated. Stains for fat and glycogen in several instances were negative. Vacuolation was often most marked in poorly fixed specimens. In many cases attempts were made to bring out a "chromaffin" reaction in the cells. In those cases in which fresh tissue was available, the technics described by Bennett [1] were used, such as fixation in formol-dichromate solution followed by mordanting for several days in 3 per cent potassium dichromate, and in some cases by "intensification" in Fontana's ammoniacal silver solution. When only fixed tissue was available, paraffin sections cut from formalin-fixed blocks were mordanted on the slide in 3 per cent solution of potassium dichromate, as recommended by Lison.[32]

In almost all instances, tissue treated with dichromate solution had

a faint yellowish color when compared with untreated tissue. However, this was never as striking as the color developed by the adrenal medulla under similar circumstances. Also the color of the neoplastic tissue was generally not more intense than that shown by sections of various other organs (*e.g.*, liver, heart, kidney) treated in the same way. No convincing evidence of a true chromaffin reaction could be found. The nonspecificity of the reaction has been commented upon above.

### *Assay for Epinephrine*

Assays * for epinephrine were carried out on 2 of the tumors. In each case the fresh tissue was frozen solid within 2 or 3 hours after surgical removal and kept in that state until the assay was performed. This was done by extracting the tissue with 0.01 N hydrochloric acid and comparing the effect of this extract with a known solution of epinephrine. Tests were done on the blood pressure of the spinal cat and on the isolated strip of ileum of the rabbit. The first tumor (no. 76947) showed an activity equivalent to less than 8 γ of epinephrine per gm. of tissue, *i.e.*, a negligible amount. The second (no. 87076) was found to have an epinephrine-like action corresponding to about 0.4 mg. per gm. on the basis of blood pressure tests and 0.25 mg. on the basis of the test with the strip of ileum. The cause of the discrepancy between the two tumors was not apparent. Further tests on the second tumor, however, indicated that the pressor substance in question was probably not epinephrine.

### *Nerve Endings*

An abundance of nerve endings has been demonstrated in the normal carotid body.[7,8] Apparently, nerve endings are absent in the tumors, although few attempts have been made to demonstrate them.[8] In one case in the series here reported, fresh tissue was fixed in 25 per cent chloral hydrate and impregnated according to one of the methods of DeCastro.† In one of several sections a few ill defined club-shaped structures were seen, but no definitely recognizable nerve fibers or nerve endings.

### *Evidence of Malignancy*

None of the tumors showed evidence of malignancy. As noted above, practically all of the tumors were described as well encapsulated and as having a fairly smooth surface. Although many of the tumors were intimately adherent to the carotid arteries, no definite invasion of sur-

* I am grateful to Drs. Otto Krayer, Ralph Brauer, and Harriet M. Maling of the Department of Pharmacology of the Harvard Medical School for these assays.

† I am indebted to Dr. James Goddard for advice concerning this technic.

rounding structures was recorded in any case. In 5 cases, lymph nodes, varying in number from 1 to 6, were removed with the tumor mass. None showed microscopic evidence of invasion by neoplastic cells. No evidence of distant metastasis was present in any case.

Mitotic figures were not seen in any of the tumors. In 8 of 17 cases there was fairly marked variation in nuclear size (Fig. 14), with occasional giant forms.

## Discussion

The ideas of Kohn [25-27] on the origin, structure, and function of the carotid body have dominated almost all discussion of the organ down to the present day. The important work of Hollinshead,[19-22] outlined briefly above, has been overlooked by even the more recent writers on carotid body tumors, with the notable exception of Bloom.[3] The physiologic evidence provided by Heymans and Bouckaert [18] and Schmidt and Comroe [41] has likewise not been emphasized. It seems clear from the work of these investigators that the carotid body is a chemoreceptor, not a gland of internal secretion; that its embryologic origin, while not entirely established, is probably not primarily from sympathetic elements; that it probably does not give a true chromaffin reaction, at least in man; that its innervation is primarily sensory; and that it does not secrete epinephrine.

There would appear, likewise, to be no satisfactory evidence that the tumors of the carotid body are derived from tissue of sympathetic origin. They resemble only superficially the pheochromocytomas of the adrenal medulla, and do not resemble at all the neuroblastomas of sympathetic origin.* Efforts to demonstrate the chromaffin reaction have been scanty and unconvincing. Most writers have been limited to formalin-fixed tissue and have regarded it as unsuitable for bringing out the chromaffin reaction, although in fact it is not.[32] Painstaking attempts like those of Bloom [3] (who failed to demonstrate the reaction) have been the exception rather than the rule. Likewise, efforts to demonstrate epinephrine by bio-assay or by chemical methods have met uniformly with failure, although a substance having a depressor effect on the blood pressure has been found [5] and in one of the tumors here reported a pressor substance of unknown nature was encountered (see above).

As for *nomenclature*, it would appear, in the light of available evidence, that terms such as "chromaffinoma," [14,34] "paraganglioma,"[16] and "adenoma" [11] are of doubtful propriety. The question whether

* The report by Cragg [6] of a carotid body tumor occurring simultaneously with tumors of the organs of Zuckerkandl is of great interest but can hardly be regarded as providing much evidence regarding the nature of carotid body tumors.

these tumors are to be regarded as hamartomas is raised by Kaufmann and Ruppanner,[23] and dismissed by the same authors because of the failure of investigators to demonstrate nerve endings in the tumors. The evidence in favor of the term "perithelioma" is discussed by Ewing.[10] However, the desirability of referring to the constituent cells as occurring "around blood vessels" when actually it is the blood vessels which surround nests of the "chief" cells, is open to question. As noted above, these cells probably may assume a spindle-like or flattened form resembling endothelium because of mechanical factors. Certainly they may, and do, take on a globular epithelium-like appearance in many instances. Also, the embryologic evidence is not in favor of an origin of these cells from endothelium or from adventitial cells in the walls of blood vessels.[4] The stroma of these tumors is admittedly exceedingly vascular, but, even in the "angiomatous" type described above, there seems to be no good reason for regarding it as essentially neoplastic or as differing in any important qualitative way from the stroma of other tumors. On the whole, it would appear that, unless the "chief" cells of the carotid body can be shown to be of the same nature as the "pericytes" of Zimmermann [47] and Stout,[35, 44, 45] the name "perithelioma" is of doubtful value. The suggestion of Bloom [3] that these tumors be designated by the noncommittal term "carotid body tumor" seems to be sound.*

Evidence of malignancy was, as noted above, singularly absent in the present series. Most of the tumors were well encapsulated, without evidence of invasion of adjacent structures, and none of the lymph nodes removed showed any tumor.†

It appears that the incidence of malignancy in these tumors may be overemphasized in the literature. I disagree vigorously with the thesis that 50 per cent of these tumors may be classified as malignant on histologic grounds.[17] While it is true that considerable variation in nuclear size, with the presence of giant forms, may occur (Fig. 14), such nuclear changes may be seen in other notably benign tumors, *e.g.*,

* Tumors of the homologous aortic bodies have not been reported in man, but Bloom [3] described two instances in dogs resembling the carotid body tumors of man. Following the report of Rosenwasser [40] of a tumor of carotid body type in the middle ear presumably arising from the "glomus jugulare," two other cases have been reported.[24, 31] These tumors may cause extensive destruction of the mastoid bone and petrous ridge.[24, 39] and one has apparently involved lymph nodes.[46]

† The only histologically malignant tumor of possible carotid body origin that I have seen is one sent by Dr. Paul Brindley of the University of Texas. It is a highly anaplastic sarcoma involving the region of the carotid bifurcation and, although its location is compatible with origin from the carotid body, the cells are so poorly differentiated that definite conclusions concerning histogenesis are impossible. In some fields multinucleated giant cells suggestive of myosarcoma are seen. As noted above, middle ear tumors of carotid body type may be at least locally invasive.

parathyroid adenomas. Mitotic figures are certainly the exception rather than the rule, and this is borne out by the frequency of prolonged clinical duration (up to 15 years in the present series).

Not a single case of visceral metastasis has been reported. (The case of Gilford and Davis [13] does not seem acceptable because of the lack of adequate illustrations.) The possible cerebral metastases recorded by Harrington *et al.*[17] are extremely questionable in the absence of autopsy evidence, and in view of the notoriously high incidence of cerebral sequelae following ligation or damage to the carotid arteries. Histologic evidence is notably lacking also in many cases in which the tumor is said to have recurred locally or to have extended upward to the base of the brain. The rate of postoperative recurrence is stated variously, being usually very low, but in some reviews fairly high.[38]

It is stated repeatedly in the literature that metastases to regional lymph nodes may occur. When, however, one attempts to find specific instances, the extreme rarity of metastasis becomes apparent. In an extensive, although not exhaustive, search of the literature, I was able to find only 2 cases [28,29] of histologically verified metastases in lymph nodes. In at least one of these,[28] the involved nodes were described as adjacent to the lower pole of the tumor and as being "infiltriert," apparently by direct extension.

The operative mortality is extremely high (about 30 per cent in the present series as well as others), due to the frequent necessity of ligating the carotid arteries in order to remove the tumor. In view of the clinically benign course of most of these tumors and the remarkable absence of symptoms in almost all cases, it would seem inadvisable to attempt surgical removal if it is necessary to ligate the carotids (admittedly it is sometimes impossible to gauge the feasibility of removal without ligation until the operation has progressed to such a point that it must be completed). The warning of Bevan and McCarthy [2] would seem to be still valid:

> "With this evidence [about 30% operative mortality], we wish to present the conclusion that in the future neoplasms of the carotid body should not be removed when it is necessary to ligate the carotid arteries in order to complete the operation. If the common carotid and the internal carotid can be saved by careful dissection, done best under local anaesthesia, the removal of a benign tumor of the carotid gland would be justified. If the surgeon had definite and satisfactory evidence that the tumor was malignant, the huge 30 per cent mortality involved in the ligation of the carotid arteries might be accepted in order to save the patient from death and from malignant disease."

I would add that such "definite and satisfactory" evidence of malignancy is rarely available at the time of operation, or, indeed, subsequently.

## Summary

1. According to present evidence, the carotid body is a chemoreceptor, not a gland of internal secretion, and not part of the "chromaffin system."

2. In a series of 17 tumors of the carotid body no true chromaffin reaction was demonstrated, and no evidence for the secretion of epinephrine was obtained in assay of the fresh tissue in two instances.

3. Tumors of the carotid body exhibit a basic pattern of nests of "chief" cells surrounded by a more or less vascular stroma. Depending on the relative amounts of "chief" cells and stroma, they may be described as "usual," "adenoma-like," or "angioma-like."

4. Use of the non-committal term "carotid body tumor" is preferred to other names generally used.

5. The great majority of these tumors are both histologically and clinically benign. In view of the high operative mortality it is doubtful whether they should be removed in those cases in which ligation of the carotid arteries is necessary.


I am indebted to Drs. Shields Warren and Olive Gates for their interest and for making available the material on which this paper is based.

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## DESCRIPTION OF PLATES

### PLATE 60

FIG. 1. Normal carotid body. Phosphotungstic acid hematoxylin stain. × 400.

FIG. 2. Normal carotid body. In this instance the cells are better preserved and plumper than in Figure 1. Hematoxylin and eosin stain. × 400.

FIG. 3. Normal carotid body. The cell nests or "Zellballen" are clearly brought out. Same carotid body as used for Figure 2. Wilder's silver impregnation for reticulum. × 300.

FIG. 4. Carotid body tumor. The "usual" pattern, representing a slight exaggeration of the normal structure, with larger cell clusters. Phosphotungstic acid hematoxylin stain. × 400.

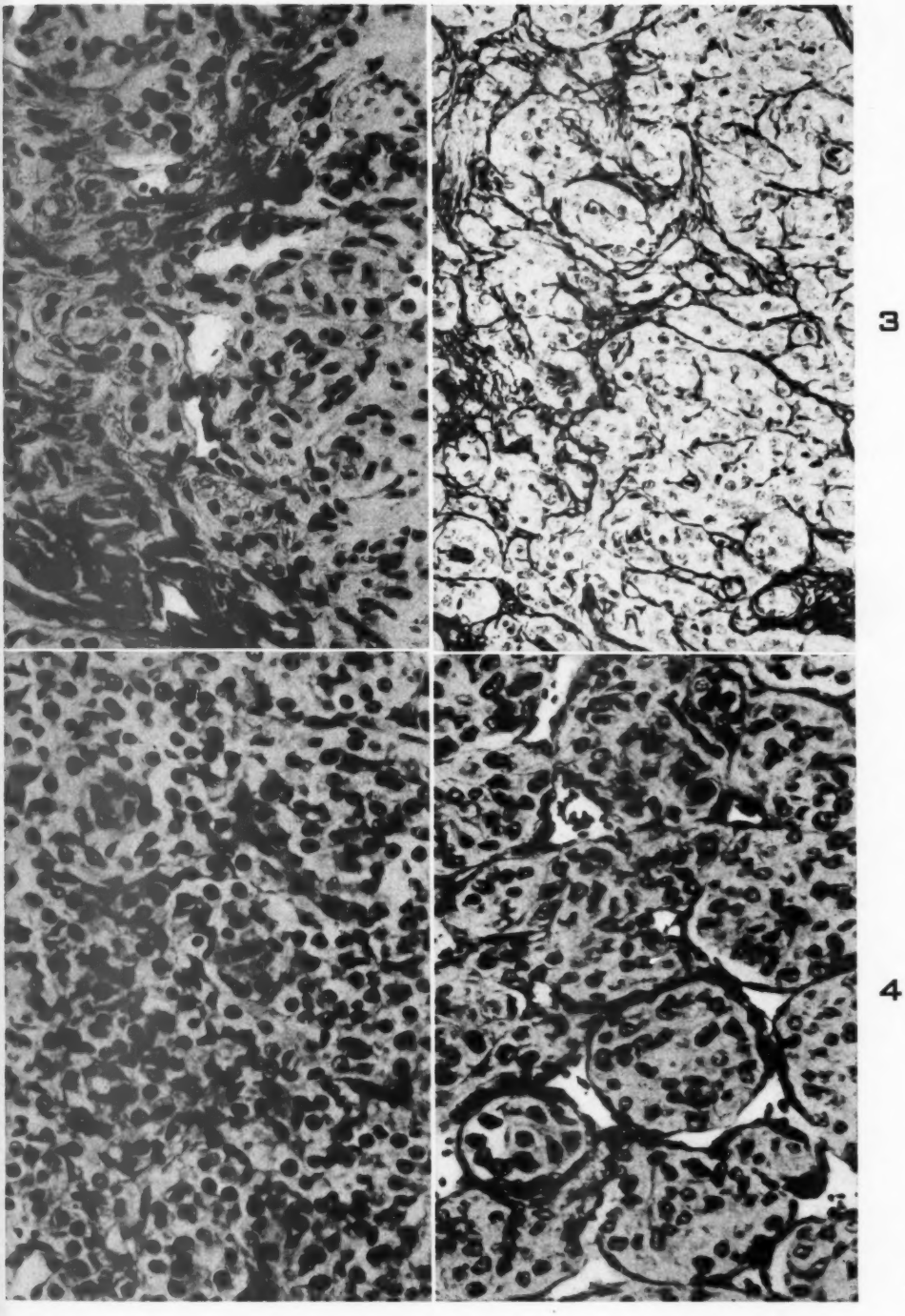


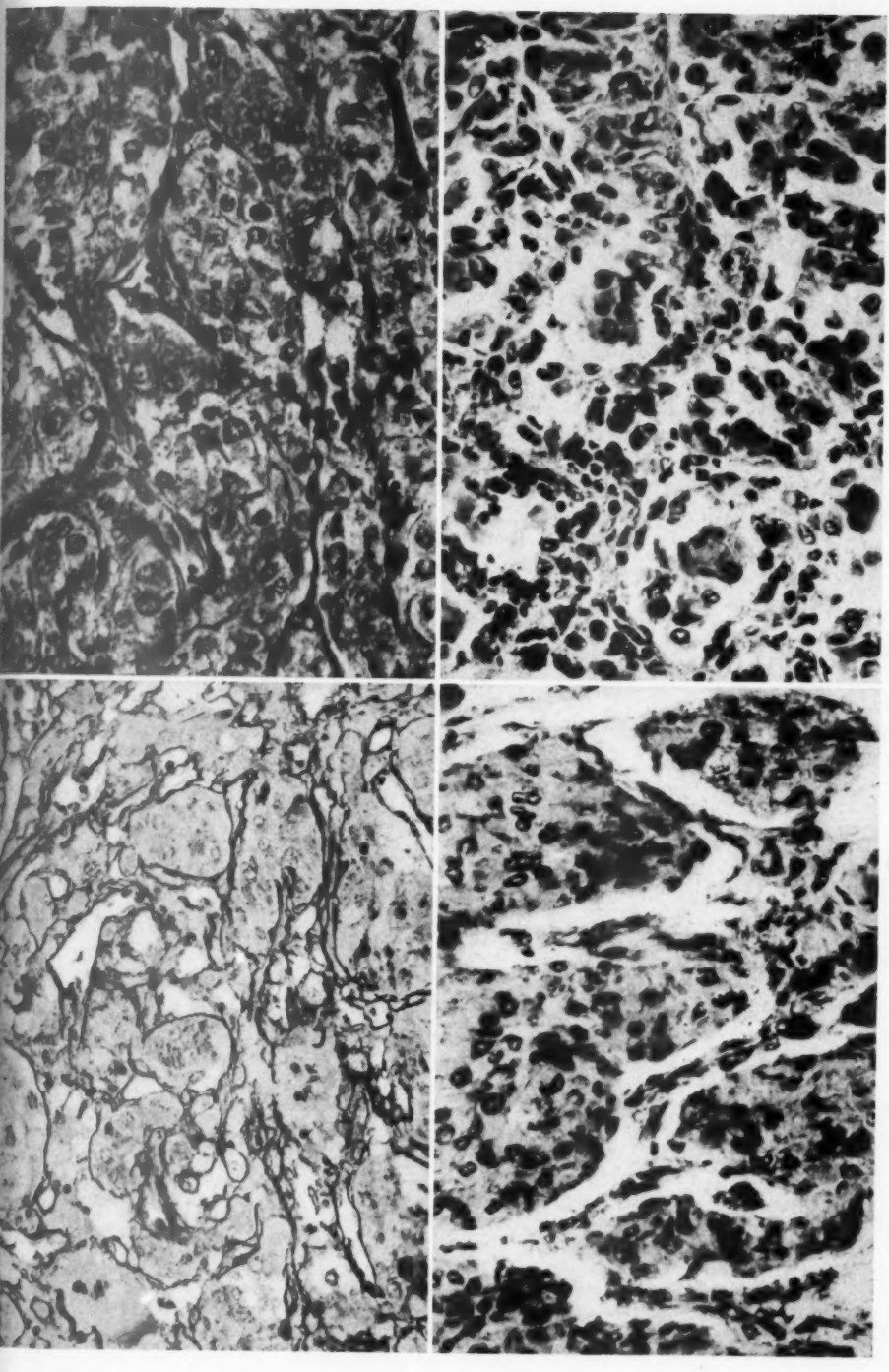
PLATE 61

FIG. 5. Another example of the "usual" type. Phosphotungstic acid hematoxylin stain. × 400.

FIG. 6. The characteristic reticulum pattern of a carotid body tumor. Same case as used for Figure 5. Foot-Bielschowsky stain. × 300.

FIG. 7. A variant of the "usual" pattern with somewhat smaller cell nests. Some of the dark material in the stroma is hemosiderin. Phosphotungstic acid hematoxylin stain. × 400.

FIG. 8. Another variant of the "usual" pattern. Rather poor preservation of the chief cells, with a tendency to elongated shape, perhaps an artifact due to compression. Phosphotungstic acid hematoxylin stain. × 400.



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8

LeCompte

Tumors of the Carotid Body

PLATE 62

FIG. 9. Reticulum pattern of the tumor shown in Figure 8. The stroma is somewhat more abundant than usual. Wilder's silver impregnation. × 300.

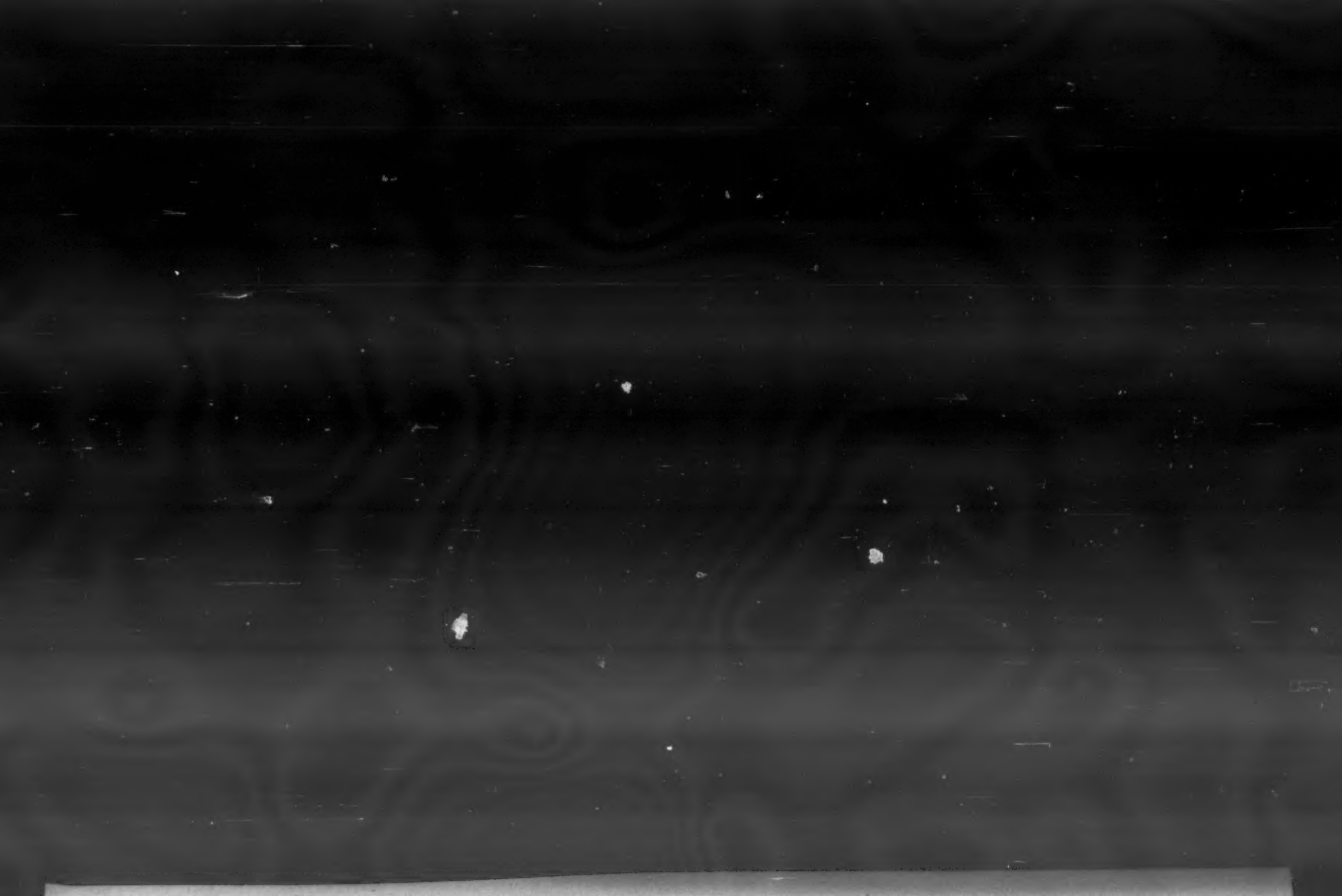
FIG. 10. The "adenoma-like" pattern. The chief cells have a strikingly epithelial appearance, but the arrangement of the "Zellballen" is maintained. Hematoxylin and eosin stain. × 400.

FIG. 11. A variant of the "adenoma-like" type. The chief cells are arranged in large sheets. Phosphotungstic acid hematoxylin stain. × 400.

FIG. 12. The "angioma-like" pattern. Here the chief cells are ovoid or spindle-shaped, and capillaries are numerous. Eosin and methylene blue stain. × 400.

FIG. 13. Silver impregnation (Wilder) of the tumor shown in Figure 12, showing that the fundamental structure is maintained, despite the angiomatous appearance. × 300.

FIG. 14. A tumor showing unusually marked variation in nuclear size, with one giant form. Hematoxylin and eosin stain. × 400.



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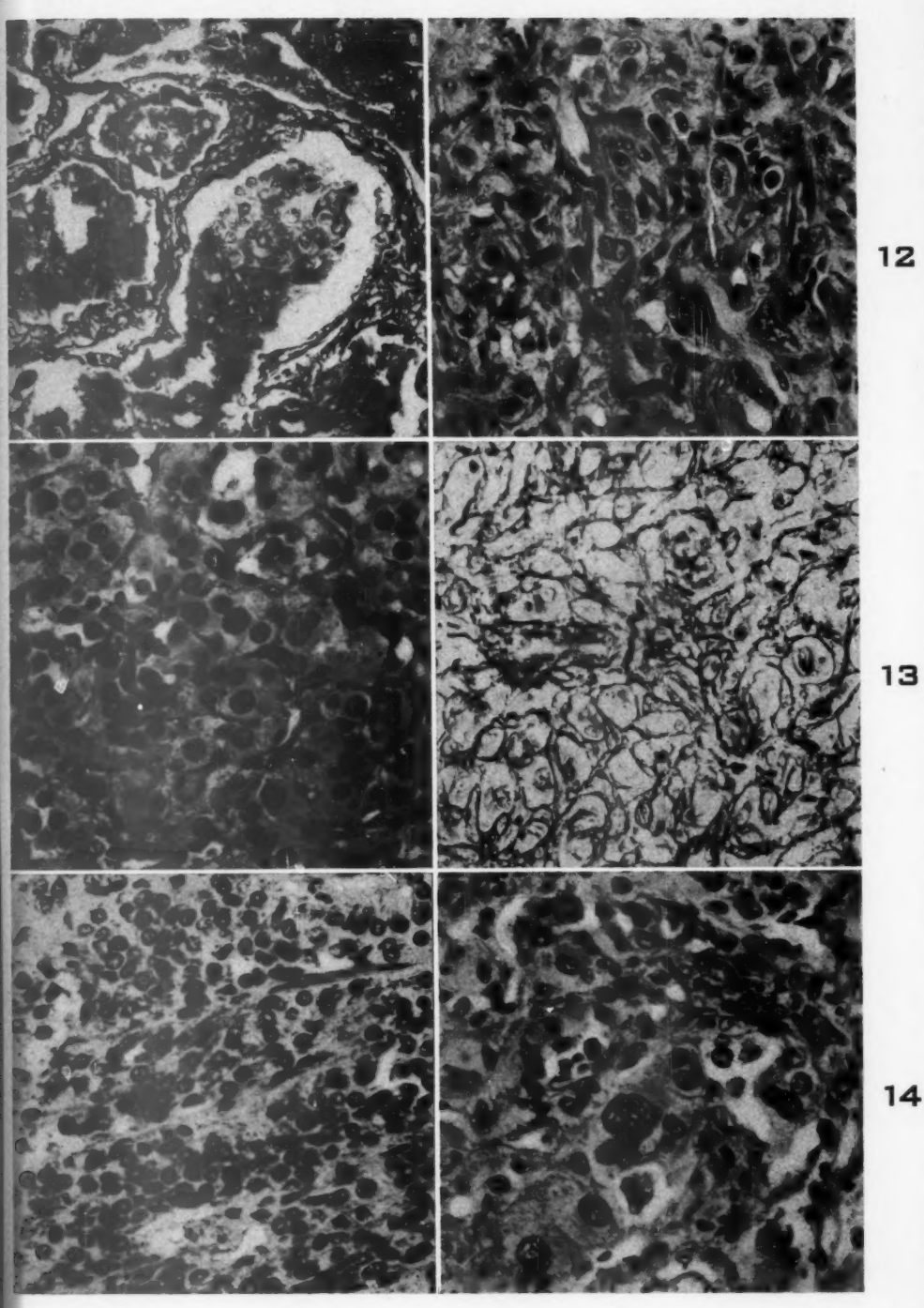

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# PARACOCCIDIOIDAL GRANULOMATOSIS
## Cardiac Localization in a Case of Generalized Form *

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Moore [1] stated that "the term blastomycosis in its medical interpretation has come to denote a more or less definite clinical syndrome with a multiplicity of causative agents." However, by reserving the use of the term "blastomycosis" for those mycoses caused by yeast-like cells reproducing in the tissues by budding or gemmation, a great advance would be attained. As Moore said, typical blastomycoses are the infections caused by fungi of the genera Zymonema, Paracoccidioides, and Cryptococcus.

In a broad sense, there is a certain geographic distribution of varieties of the disease, justifying the designations "North American blastomycosis" (caused by the Blastomyces or *Zymonema dermatitidis*), "European blastomycosis" (*Cryptococcus neoformans—Torula histolytica*), and "South American blastomycosis" (*Paracoccidioides brasiliensis*). However, each of these denominations should be reserved for the prevalent or endemic form of the disease in a particular region, without excluding the possibility of the existence of rare, sporadic cases of the other varieties of blastomycosis.

In Brazil, as well as in other South American countries, the prevalent form of blastomycosis is the disease of Lutz-Splendore-Almeida, produced by the *Paracoccidioides brasiliensis* (Splendore), established by Almeida in 1930. Lutz [2] (1908) was the first to identify the disease in Brazil, and in the same year Carini [3] reported the second case, with the same localization of lesions as in the case of Lutz. In 1909, Splendore started a series of publications on the mycosis of Lutz, and considered [4] (1912) the causative agent as of the genus Zymonema (De Beurmann and Gaugerot), species *brasiliensis*, n.sp.

From then until 1929, there followed a period of incomprehensible disagreement among research workers, during which the chief tendency was to attempt to identify Brazilian blastomycosis with North American coccidioidal granuloma. That tendency is the more surprising when it is considered that Lutz and Splendore described the fungus as reproducing in the tissues by budding. As a matter of fact, in Lutz's first reference the disease was classified as a "pseudococcidioidal" mycosis, differing therefore from the coccidioidal granuloma

* Received for publication, March 26, 1947.

described by Rixford and Gilchrist [5] in the United States in 1896, the agent of which reproduces characteristically by endosporulation.

The chief works that have contributed to the confusion are as follows: Vianna[6] stated that he always found parasites multiplying by endosporulation, and only rarely by budding. Carini [7] described the reproduction of the fungus as being by budding, not endosporulation, and yet concluded inexplicably that the parasite is the *Coccidioides immitis*. Pedroso [8] was of the opinion that the fungus reproduces by endosporulation, and classified it as *Coccidioides immitis*. Haberfeld [9] wrote an interesting paper denying peremptorily reproduction by budding for the agent of Brazilian blastomycosis; he supported the view that its only form of reproduction is endosporulation, and classified the fungus as *Zymonema histosporocellularis*. Finally, Fonseca and Area Leão [10] studied one case of Brazilian blastomycosis and described the reproduction of the fungus as being exclusively by endogenous sporulation, the spores being liberated through small openings in the membrane, but continuing to be attached to the mother cell for some time by a protoplasmatic filament. They concluded, therefore, that the fungus is identical to *Coccidioides immitis*, found by Rixford and Gilchrist [5] in the United States.

In 1927, Souza Campos and Almeida [11] were the first to make a comparative study of Brazilian and North American blastomycoses, having had 12 cases of the former and 2 of the latter variety; they were then able to draw conclusions as to their diversity. Proceeding in that comparative study, Almeida [12] established in 1929 that the causative agent of Brazilian blastomycosis is a new species of the genus Coccidioides. In 1930, Almeida [13] finally was able to establish a new genus—Paracoccidioides—for that organism, based on abundant material and experimental work, separating it definitely from the genus Coccidioides.

Coming to Brazil to study Almeida's new genus, Moore [1] not only confirmed those findings but also described two new species, *Paracoccidioides cerebriformis* and *P. tenuis*.

From the clinical point of view as well as from that of pathogeny, there are fundamental differences between the North and the South American blastomycoses. According to Conant and co-authors,[14] in the North American variety the prevalent localizations are in the skin, lungs, and bones, the lesions being of the suppurative or granulomatous type. The organisms enter the skin or the respiratory mucous membrane. In the Brazilian form, primary cutaneous lesions are exceptional, if existent at all; they usually represent foci of secondary dissemination.[15] In almost all cases, the fungus penetrates the bucco-

pharyngeal mucous membrane,[16] giving rise to painful infiltrative ulcerations, with the papillomatous or vegetative aspect of stomatitis ulcerosa moriformis (mulberry-like) [15] in the gums, floor of the mouth, lips, cheeks, soft palate, uvula, and palatine arches, with occasional extension to pharynx and larynx. The tonsils [16] are usually involved primarily or secondarily with the lesions either visible or not at the surface; the latter circumstance explains some rare cases of cervical paracoccidioidal adenopathy with no apparent lesion in the buccopharynx.

The primary lesions are followed by a rapid lymphatic dissemination with involvement of the cervical lymph nodes. These are gradually enlarged, and the lymphadenitis may evolve to softening and fistulization, or to fibrosis and induration.[17] This lymphatic dissemination may progress to other groups of lymph nodes, namely, the supraclavicular or axillary. This form, with involvement of integument and secondary regional lymphatic dissemination, constitutes the lymphaticotegumental form. * In the great majority of cases these lymphaticotegumental manifestations represent an evolutive phase of the infection, which tends to become systemic by spreading through the blood stream. This may happen after 3 to 18 months, with the development of visceral and cutaneous lesions accompanied by polyadenopathy, leading to cachexia and death.

In the systemic form of visceral generalization any organ may be involved, the more frequently affected being the spleen,[18] liver, intestines,[19] pancreas, and lungs [20]; since these lesions are accompanied by intense involvement of the regional lymph nodes, this is said to be the lymphaticovisceral form.

Finally, in the terminal stages of the infection the lesions are multiplied through lymphatic and hemal dissemination, constituting the mixed forms, in which the disease spreads to the whole body, and may affect any organ.

## Report of Case

### Autopsy Findings

Autopsy was performed upon the body of a 38-year-old Japanese (height, 151 cm.; weight, 42 kg.) in an advanced state of cachexia, who had had no medical assistance.

The face showed an extensive ulceration of the skin covered by a muddy crust; similar smaller lesions were found in the left eyebrow, lobule of the right ear, lateral aspects of the neck, supraclavicular fossae, axillae, inguinal regions, and thighs. The pharynx, epiglottis,

* Tegumental is here used in its broad sense, that is, "pertaining to the tegument," both cutaneous and mucosal.

and larynx showed multiple irregular ulcerations of various sizes. The tonsils were extensively destroyed. The lungs showed multiple hard whitish areas, especially in the inferior lobes. Hydrothorax (500 cc.) and pleural adhesions were found on the left side. Macroscopically, the heart seemed normal. The abdominal cavity contained about 600 cc. of a turbid serofibrinous exudate, the intestinal loops being covered by deposited fibrin. Several yellowish, hard, miliary nodules were found in the liver, as well as in the spleen and cortical substance of the kidneys. No lesions were observed in the pancreas. The lymph nodes showed lesions varying in size and consistence, but mostly soft caseous areas. The microscopic study of smears of this material revealed the paracoccidioidal fungus in large numbers.

*Anatomic Diagnoses.* Serofibrinous peritonitis; paracoccidioidal granulomatosis of the tonsils, pharynx, epiglottis, larynx, lungs, liver, spleen, kidneys, lymph nodes, and skin.

### *Microscopic Findings*

*Myocardium.* Slide 1 (Fig. 1). Inside a myocardial fiber there were a large number of young paracoccidioidal fungi, each measuring 5 to 6 $\mu$ in diameter. This conglomerate of small parasites occupied a large part of the length of the muscle fiber and completely destroyed its structure. There was no evidence of adjacent inflammatory reaction. In another field (Fig. 2), there was a single adult fungus inside a muscle fiber, the structure of which was destroyed. Again, no inflammatory reaction was noted in the vicinity.

Slide 2 (Fig. 3). A nodular, poorly limited area was seen in a bundle of muscle fibers, where the structure was altered by the partial destruction of the fibers. The central zone of this area showed a paracoccidioidal fungus in multiple gemmation surrounded by a characteristic marginal crown of spores. Among the tissue débris and around the organism there was a cellular reaction represented by some mononuclear lymphocytoid cells and a few polymorphonuclear leukocytes. This is a later stage of the process seen in slide 1, showing a tendency towards the formation of a nodule of reaction.

Slide 3 (Fig. 4) showed a rarefied, oval, well circumscribed area of destroyed muscle fibers, in which there was some amorphous débris of the fibers and two adult parasites, together with a nodular conglomerate of epithelioid cells, a few lymphocytes, and some polymorphonuclear leukocytes. There were no giant cells.

Slide 4 (Fig. 5) showed an advanced stage of the process. The lesion in the muscle bundle appeared as a rarefied, well circumscribed area of destroyed muscle fibers, in the débris of which there were free

parasites. The area showed also a cellular proliferation represented mainly by histiocytes together with elongated cells having fusiform or oval nuclei, that is, with the characteristics of fibroblasts. There were a few lymphocytes, chiefly at the periphery of the area, but no giant cells.

Slide 5 (Fig. 6). There were two adult fungi, close together, in the interstitial tissue between two muscle bundles. In close contact with the capsule of the parasites there were several elongated nuclei, and in their immediate vicinity there was a moderate cellular reaction represented by lymphocytoid elements and a few fibroblasts.

*Epicardium.* In the epicardium two cellular nodules were found containing fungi and formed by a reactionary proliferation of histiocytic mononuclear cells and small lymphoplasmacytic elements. In one of the nodules, around the parasite, a poorly limited acidophilic mass was seen containing four small adjoining nuclei, suggesting the formation of a giant cell. Both nodules were deeply situated in the serosa, close to the myocardium. In the lumen of an epicardial vessel (Fig. 7), several paracoccidioidal fungi were seen free in the circulating blood; one of these was in the process of reproduction.

*Lungs.* The lungs showed nodular structures in the alveolar walls, projecting to various degrees into the lumina of the alveoli. The structures were constituted by a proliferation of histiocytes, one or more giant cells, lymphocytes, and plasmacytes. As a rule, the nodules contained large numbers of parasites, the majority of which were phagocytized; many of them were in reproduction. Close by, in the interstitial tissue, there was a similar granuloma at the side of a vessel. In other fields the alveolar lumina were free, while the walls were thickened by a marked dilatation of the congested capillaries. In neighboring areas the alveolar lumina were occupied by a homogeneous acidophilic substance containing erythrocytes and large, rounded mononuclear cells. In this massive zone, some irregularly distributed alveolar lumina contained a cellular conglomerate of large epithelioid cells together with one or more multinucleated giant cells phagocytizing parasites, some of which were in reproduction.

*Lymph Nodes.* The normal structure of the lymph nodes was profoundly altered. There were extensive irregular areas, isolated or confluent, formed by poorly stained débris of disintegrated tissue, containing a large quantity of free parasites, some of which were in reproduction. There remained only a few small conglomerates of lymphocytes and lymphadenoid tissue, mostly at the periphery of the node, where small granulomas were found containing parasites included in giant cells and histiocytes.

*Liver*. Numerous well circumscribed granulomas of various sizes were disseminated throughout the liver. They consisted of epithelioid histiocytes, a few lymphocytes, and, in most cases, one or more giant cells phagocytizing fungi, the majority of which were in reproduction. Some of the granulomas showed an incipient fibrosis, revealed by nuclei of fibroblasts found at the periphery or inside the nodule. The granulomas were localized both inside the hepatic lobules and in the stroma of the spaces of Kiernan.

*Spleen*. The focal lesions of the spleen were found both in the lymphatic nodules and splenic pulp. Most of the malpighian bodies showed variable disorganization in their constitutive elements, which were dissociated by nodular granulomatous proliferations. The histiocytic reaction in the red pulp was extensive, and included giant cells with phagocytized fungi as well as numerous plasmacytes and a fair number of eosinophils.

*Pancreas*. The structure of the pancreas was preserved, including the islands of Langerhans. There was a slight increase in the interstitial connective tissue. A few nodular granulomas were found in the lobules (Fig. 8), showing a slight histiocytic reaction and some giant cells phagocytizing parasites.

*Kidneys*. Lesions were found both in the cortical and medullary substance of the kidneys. Several intact glomeruli showed a small number of free parasites in the lumina of the capillary loops. In Figure 9 an organism is seen entering the afferent arteriole. When the number of parasites was high, the glomeruli showed varying degrees of necrosis, due to direct toxic action of the fungi; when that number was low, the glomeruli remained intact, and no periglomerular reaction was seen (Fig. 10). In the damaged glomeruli Bowman's capsule was partially destroyed and there developed a periglomerular reaction with numerous lymphocytes, some neutrophils, and mononuclear cells. This exudate sometimes included a parasite which had migrated through the ruptured Bowman's capsule (Figs. 11 and 12). The lumina of the convoluted and straight tubules showed varying numbers of parasites. When their number was low, there was no reaction in the vicinity; but when fungi were numerous (sometimes they filled the tubular lumen) the wall might be destroyed, and there was an adjacent inflammatory reaction similar to the one observed around the damaged glomeruli (elimination form).

The preparations also showed disseminated areas of variable size, mostly in the cortical substance where the normal structure was replaced by a granulomatous tissue, representing a more advanced stage of the process. They consisted of a proliferation of histiocytes, numer-

ous giant cells with phagocytized parasites, lymphocytes, plasmacytes, and some eosinophils, found mostly in the peripheral zone of the lesion. In some of these areas the core was occupied by densely packed neutrophils, the majority of them degenerated, while in other lesions the central portion was invaded by fibroblasts.

*Skin.* The skin showed two distinct lesions. One was localized in the dermis, extending out to the papillary layer, and consisted of two juxtaposed nodular areas. These areas showed a dense conglomerate of parasites destroying the pre-existing structures, surrounded by a coat of parvicellular elements, predominantly plasmacytes. Many of these cells showed regressive alterations of their nuclei, mostly pyknosis. The adjacent vessels and glands were densely infiltrated, mostly by plasmacytes. The covering epidermis showed no alterations.

The other lesion was more extensive. It was situated in the dermis and expanded secondarily to the epidermis. In the deeper coat it was similar to the first lesion. In the epidermis, the basal and spinous strata were destroyed, while the stratum corneum showed a marked parakeratosis and invasion of paracoccidioidal fungi, neutrophils and, at the surface, colonies of bacteria. The neutrophils extended secondarily to the dermis, chiefly to the papillary layer.

### *Diagnosis*

Paracoccidioidal myocarditis, pericarditis, bronchopneumonia and interstitial pneumonitis, lymphadenitis (gummatous form), hepatic granulomatosis (nodular form), splenic granulomatosis (nodular and disseminate forms), pancreatic granulomatosis, hematogenic nephritis, and dermatitis with secondary ulceration.

## Discussion

The case described presents a condition of acute paracoccidioidal sepsis, as a terminal stage of a paracoccidioidal granulomatosis. As far as could be ascertained, this is the first demonstration in histologic sections of the reproduction of paracoccidioidal fungi in the circulating blood. Besides the consequent localization of the organisms in several organs, as far as is known this is also the first report of cardiac lesions caused by this fungus.

In this case, the dissemination to the heart was hematogenic. Initially, the parasites may enter the myocardial fibers or the interfascicular connective tissue. In the former localization the parasite was found in the adult stage, as well as in its small, young forms. In both localizations there is a profound structural alteration of the muscle fiber, with destruction of myofibrils through compression or toxic action of the

parasites. This stage shows, as yet, no local inflammatory reaction to the presence of the organisms, as they are still inside the fiber and do not activate the adjacent tissues. To this initial direct lesion of regressive character there is added the destruction of the muscle fiber and liberation of organisms and débris in the interstitial tissues, where they give rise to a local reaction. This initiates the appearance of lymphocytoid cells and a few neutrophils.

In subsequent stages, mononuclear and histiocytic cells of epithelioid aspect appear and predominate; their local conglomeration constitutes the granulomatous nodule, which usually contains parasites. In some granulomas, a marginal fibroblastic proliferation develops, indicating the tendency to sclerosis of the lesion. The death of the patient possibly interrupted the progress of the process to the later stages that sometimes have been found in other organs of other patients.

The interstitial and epicardial lesions develop in a like manner.

The renal lesions reached a higher development than the ones just referred to. The dissemination to the kidney is also hematogenic, as is well evidenced in Figure 9, where the parasite is entering the glomerulus through an afferent arteriole. In the glomeruli, the fungi induce regressive lesions and necrosis of varying portions of the capillary loops, or even total destruction of the glomerulus. The lesion of the Bowman's capsule opens the way to an active periglomerular interstitial reaction. The periglomerulitis consists of a dense conglomeration of lymphocytes, monocytes, and neutrophils. Through the destruction of glomerular capillaries the fungi enter Bowman's space and are drained to the renal tubules, where they give rise to new foci, thus characterizing the form of elimination of hematogenic nephritis.

All of the multiple lesions found in the different organs show the characteristics of an acute hematogenic dissemination, including the cutaneous lesions, which arose in the dermis.

## Summary

A case of paracoccidioidal granulomatosis (Brazilian blastomycosis) is reported, terminating in an acute paracoccidioidal sepsis.

The findings may be summarized as follows:

1. *Paracoccidioides brasiliensis* was found reproducing in the circulating blood.

2. Specific myocardial and epicardial lesions are reported for the first time.

3. The renal lesions are of the hematogenic focal nephritic type.

4. The multiple lesions found in several organs, including the skin, demonstrate the acute hematogenic dissemination of the infection.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

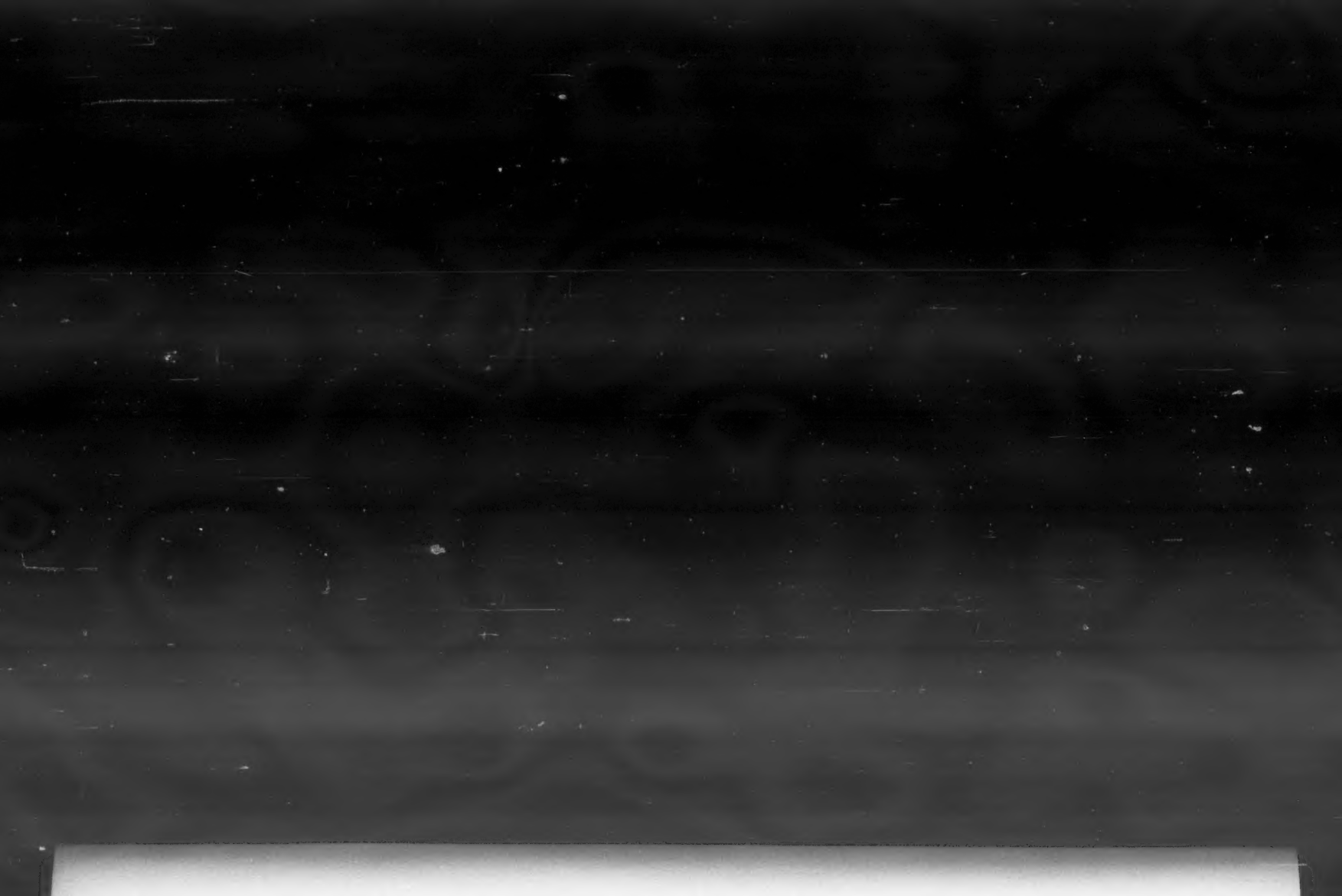
### PLATE 63

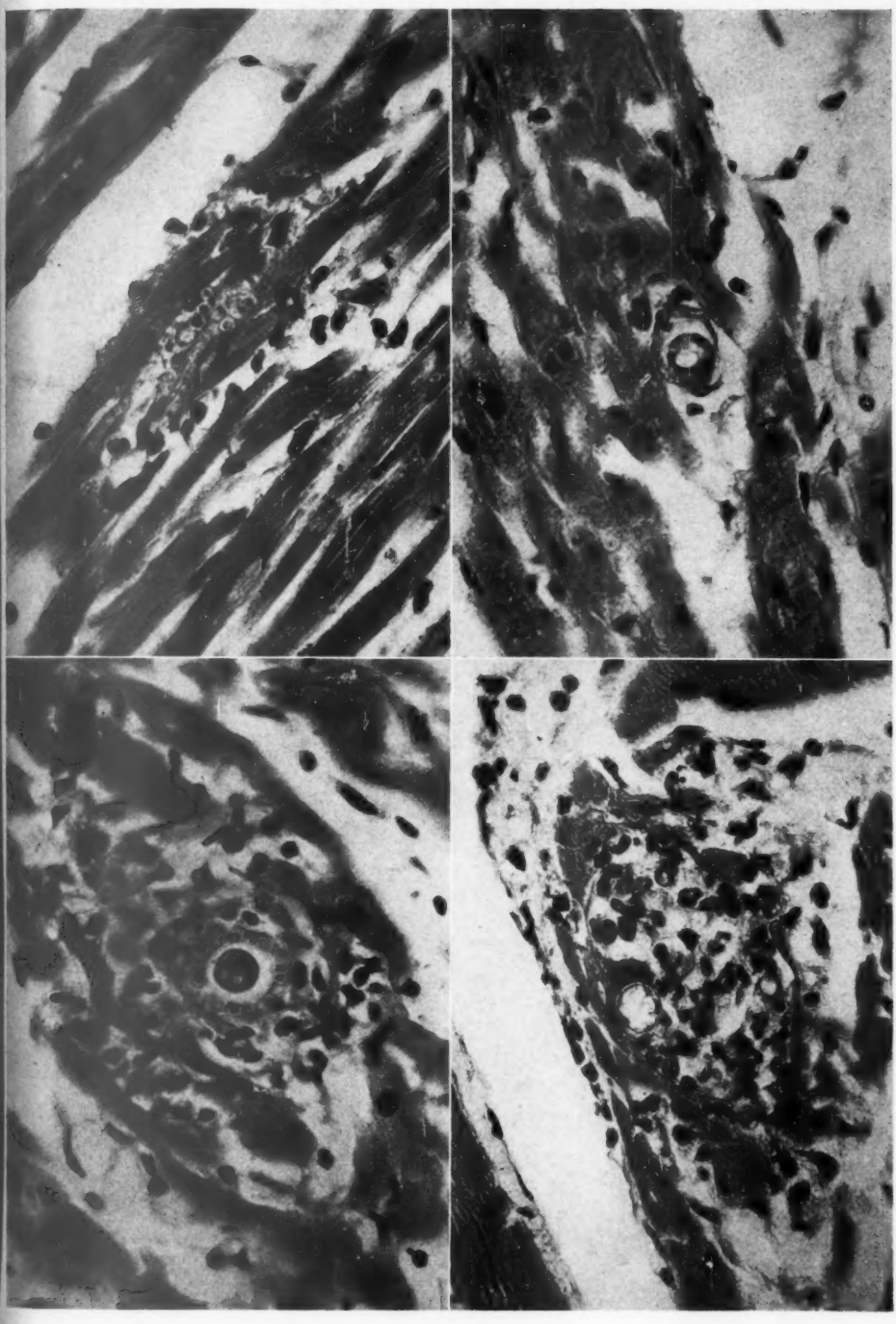
FIG. 1. Young paracoccidioidal fungi inside a myocardial fiber. Hematoxylin and eosin stain. × 950.

FIG. 2. Adult fungus inside a myocardial fiber. Hematoxylin and eosin stain. × 750.

FIG. 3. Early nodular reaction around a paracoccidioidal organism in multiple gemmation, in the myocardium. Hematoxylin and eosin stain. × 750.

FIG. 4. Nodular area of reaction containing two adult parasites, and showing destruction of the myocardial fibers, epithelioid cells, lymphocytes, and some polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 750.





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Cunha Motta Paracoccidioidal Granulomatosis

## PLATE 64

FIG. 5. Advanced stage of the process in a muscle bundle, showing destruction of myocardial fibers, free parasites, histiocytes, and initial proliferation of fibroblasts. Hematoxylin and eosin stain. × 450.

FIG. 6. Two adult parasites in the interstitial tissue of the myocardium. Hematoxylin and eosin stain. × 450.

FIG. 7. Paracoccidioides in reproduction, free in the circulating blood of an epicardial vessel. Hematoxylin and eosin stain. × 950.

FIG. 8. Granuloma in the pancreas, containing a parasite in reproduction. Hematoxylin and eosin stain. × 185.

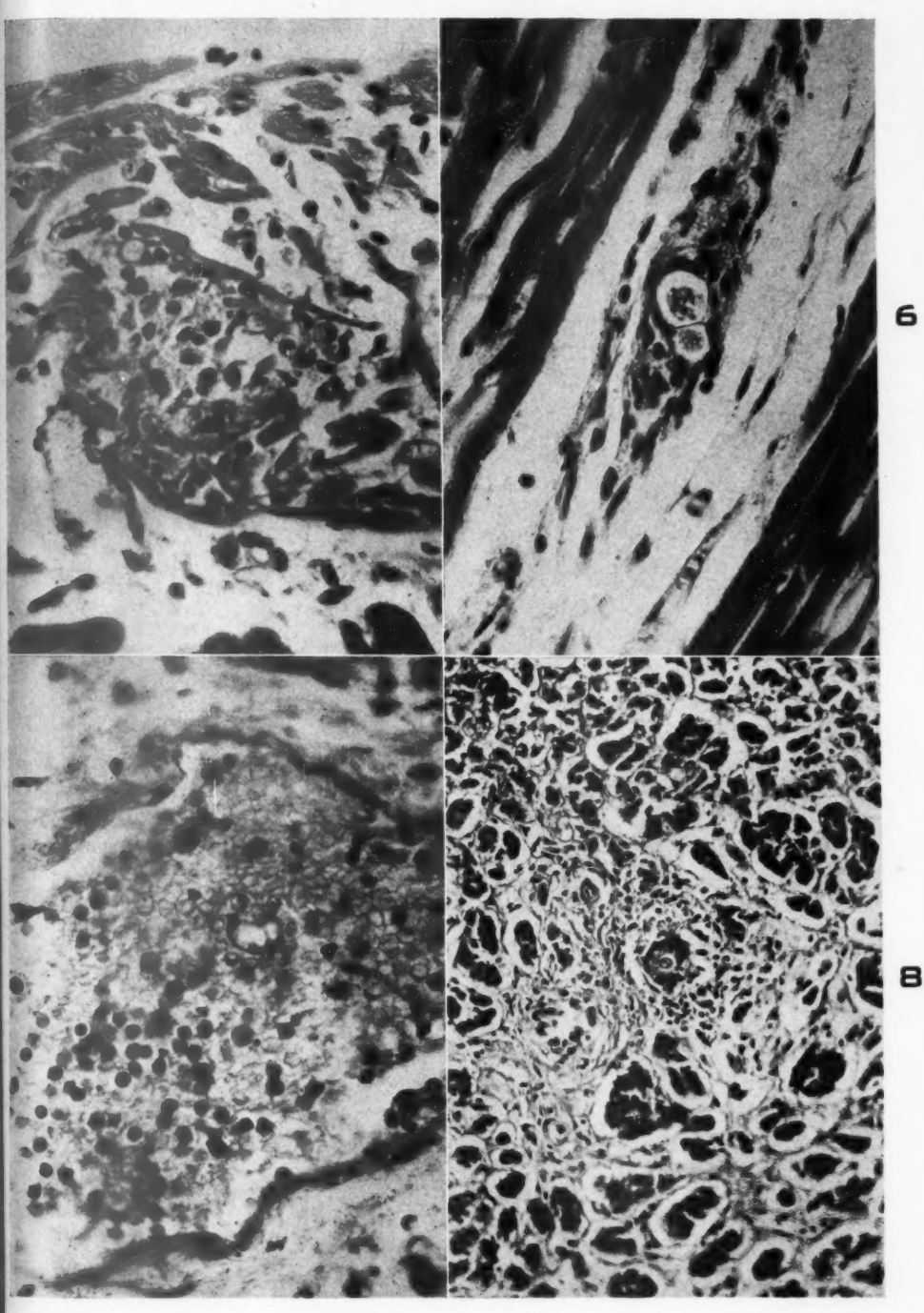
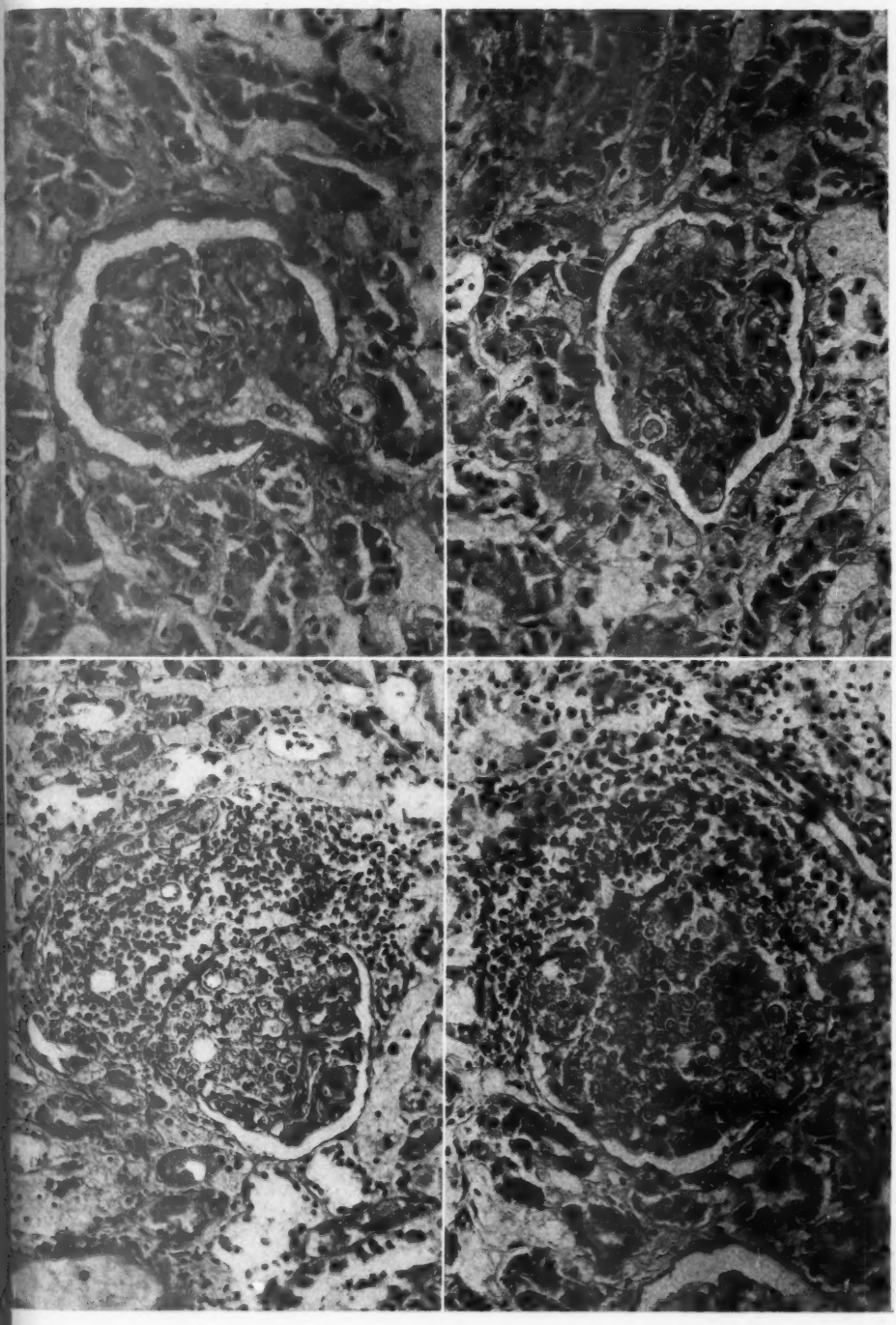


PLATE 65

FIG. 9. Paracoccidioides free in the blood of an afferent glomerular arteriole. Hematoxylin and eosin stain. × 500.

FIG. 10. Single parasite in a glomerulus. No periglomerular reaction is seen. Hematoxylin and eosin stain. × 500.

FIGS. 11 and 12. Glomerulus with numerous parasites, showing rupture of Bowman's capsule with periglomerular reaction. In the exudate there are also parasites migrating through the ruptured Bowman's capsule. Hematoxylin and eosin stain. × 230.



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Cunha Motta Paracoccidioidal Granulomatosis

# SYSTEMIC NORTH AMERICAN BLASTOMYCOSIS
## REPORT OF A CASE WITH CULTURAL STUDIES OF THE ETIOLOGIC AGENT AND OBSERVATIONS ON THE EFFECT OF STREPTOMYCIN AND PENICILLIN IN VITRO *

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North American blastomycosis is a fungus disease caused by *Blastomyces dermatitidis*. It is manifested in two clinical forms, cutaneous and systemic. The former, beginning as a small pimple or pustule on the hands, face, wrists, ankles, or other exposed site, or in an area injured by abrasion, proceeds to a chronic or subacute ulcerating lesion of the skin. Skin lesions of cutaneous blastomycosis are characteristically single, spread slowly and peripherally, and may cause extensive involvement with no evidence of visceral infection. The cutaneous form of the disease is seldom fatal and usually responds to treatment with iodides and x-ray therapy. Systemic blastomycosis, on the other hand, is a fatal disease ordinarily characterized by pulmonary involvement and widely disseminated lesions in the subcutaneous tissues, bones, joints, central nervous system, and internal organs.[1] In contrast with the cutaneous type, skin lesions in this form of the disease are multiple instead of single and are widely distributed over the skin on unexposed parts of the body such as the abdomen, back, and thighs. Both cutaneous and systemic manifestations of blastomycosis are caused by the same fungus.

The characteristic reaction of the tissues to the organism in either systemic or cutaneous blastomycosis is abscess formation with chronic granulomatous inflammation, giant cells, necrosis, and fibrosis. Numerous abscesses of almost microscopic size are found in skin lesions, while old lesions show signs of chronic inflammation and scarring. There is extensive epithelial hyperplasia about the margins of the ulcerating skin lesions. Although the tissue reaction in the systemic form of the disease is usually pyogenic, the presence of caseation necrosis and tubercle formation may cause it to be confused with tuberculosis.[2] Nevertheless, the tissue reactions in blastomycosis and tuberculosis are not identical,[3] the most obvious difference being the suppurative reaction in blastomycosis. Comprehensive reviews of the subject are available in the excellent papers of Martin and Smith,[4] of Baker,[5] and

* Received for publication, May 24, 1947.

of Friedman and Signorelli.[5] Cases of North American blastomycosis have been reported almost exclusively from the United States. Proved and presumptive cases have been found in 28 states, with Illinois, Louisiana, Kentucky, Tennessee, Virginia, and North Carolina furnishing the larger numbers.

The purposes of the following report are (1) to present the clinical and pathologic features of a case of fatal systemic North American blastomycosis involving the meninges as well as other organs of the body; (2) to present and discuss the method of primary isolation and identification of *B. dermatitidis;* and (3) to describe tests of the resistance of the fungus *in vitro* to streptomycin and penicillin.

## Report of Case

A 20-year-old, colored, male laborer entered Charity Hospital on May 16, 1946, because of tender cutaneous nodules which had grown progressively larger and more painful over the preceding 3 weeks. He had been well prior to the present illness and had had no symptoms referable to the gastrointestinal, genito-urinary, or nervous systems. For a period of 3 months he had had slight afternoon fever, night sweats, and blood-streaked sputum. He had been employed as a laborer in an oil field in central Mississippi but reported no prolonged contact with irritating dusts or chemicals. The past history was otherwise noncontributory.

*Physical Examination.* The patient was lying flat in bed without dyspnea, and was oriented and alert; his respiratory rate was 23; pulse, 94; and temperature, 99.4° F. He was well developed but poorly nourished and appeared severely ill. On the left forehead there was a large encrusted lesion, measuring 2 by 3 cm., which was sharply demarcated from the surrounding normal skin. Pus could be expressed from beneath the eschar. There were no lesions of the mucous membranes. Multiple subcutaneous, fluctuant masses, painful to palpation and varying in diameter from 2 to 5 cm., were present over the arms, forearms, thighs, and legs. There was no palpable enlargement of the superficial lymph nodes. The lungs were clear to percussion and auscultation. The heart was not enlarged and no abnormal sounds were heard. There were no palpably enlarged viscera in the abdomen and no abdominal tenderness or rigidity. There was no edema of the extremities. No signs of meningismus were elicited.

*Laboratory Findings* (on admission). *Blood:* Hemoglobin, 7 gm. per 100 cc.; red blood cells, 2.75 million per cmm.; white blood cells, 15,350 per cmm. Differential blood cell count: 63 per cent polymorphonuclear leukocytes, 13 per cent immature polymorphonuclear leukocytes, 4 per cent monocytes, 20 per cent lymphocytes. Sedimentation rate (Wintrobe) was 67 mm. in 20 minutes, 78 mm. in 1 hour. The Mantoux test was negative with 1:10,000 dilution of old tuberculin. The Kline and Kolmer tests were negative. *Urine (voided specimen):* Yellow, acid; specific gravity, 1.012; albumin, negative; sugar, negative; 2 to 3 red blood cells and 5 to 6 white blood cells per high power field.

*Clinical Course.* A roentgenogram of the chest taken on admission revealed multiple infiltrations of pin-head size throughout both lung fields (Fig. 1). Since the findings were compatible with miliary tuberculosis, the patient was admitted directly to the Tuberculosis Unit. Sputum examinations (24-hour concentrations) on three occasions revealed no acid-fast bacilli. The vital capacity was recorded as 1,600 cc. The patient was febrile with sharp, irregular fluctuations in temperature up to 102° F. He showed severe muscular weakness throughout the period of observation and frequently required codeine for relief of chest pain.

Toward the end of the first hospital week in the Tuberculosis Unit, the patient became irrational and it was feared that he had developed tuberculous meningitis. A spinal puncture yielded clear cerebrospinal fluid under an initial pressure of 210 mm. of water. The fluid contained 10 cells per cmm., and 31 mg. per cent of protein. The Kline and Kolmer tests were negative; the colloidal gold test gave no reaction.

Cultures of sputum collected on May 24 showed growth of fungi after 6 days, but the organisms were not definitely identified. Pus was then aspirated from the subcutaneous abscesses and examined microscopically in a moist mount. Numerous doubly contoured bodies, measuring approximately 6 to 16 $\mu$ in diameter, were seen. Several of the organisms possessed single buds extending from the periphery of the cell. A diagnosis of blastomycosis was entertained and on the 18th hospital day the patient was transferred to the Isolation Unit for treatment.

Upon admission to the Isolation Unit, the patient was anemic, emaciated, edematous, dyspneic, and irrational. Crepitant râles and dullness were present in the base of the left lung and in the left axilla. The heart rate was rapid but regular and a systolic murmur was heard at the apex. The liver was tender and extended 3 finger-breadths below the costal margin. No other masses were felt in the abdomen. The deep reflexes were active and equal. Nuchal rigidity was present, as was Kernig's sign. The subcutaneous abscesses were still present and were quite painful. Microscopic examination of pus from the ulcer on the forehead revealed many oval, doubly contoured, highly refractile bodies which developed small peripheral buds when left at room temperature for 24 hours on a sealed slide mount.

The patient was given a high-vitamin, soft diet, and sedation as needed. He required increasing amounts of narcotics to control the pain in the chest. A transfusion of 500 cc. of whole blood and repeated infusions of normal saline and glucose solution were administered. However, he failed rapidly. His respiratory rate increased to 52 per minute and he became more irrational, experiencing visual and auditory hallucinations during the last 2 days of life. He gradually lapsed into coma, refused all food, and expired on the 22nd hospital day, approximately 4 months after the onset of symptoms.

*Clinical Diagnosis:* Systemic blastomycosis.

### *Autopsy Report*

Autopsy was performed 2½ hours after death. The body was that of a well developed, poorly nourished, colored, adult male weighing 120 lbs. and measuring 177 cm. in length. Marked pedal edema was present. Granulating ulcers of the skin with slightly raised, irregular edges and necrotic, hemorrhagic bases were present as follows: lesion, 3 cm. in diameter, on the left side of the forehead; two lesions, 3 and 1.5 cm. in diameter, respectively, on the right side of the chin; lesion, 1 cm. in diameter, under the chin; lesion on the right hip, 3 by 1 cm. in diameter. Small, firm, cervical lymph nodes were palpable on the left side of the neck. Round to oval subcutaneous abscesses varying from 3 to 8 cm. in diameter were noted in the following sites: the left side of the neck below the angle of the jaw, the midportion of the inner aspect of the right forearm, below the elbow on the medial aspect of the left forearm, and the calf of the left leg. The pus in these cavities was creamy and varied from pale yellow to greenish gray or bloody. A draining sinus, 1 cm. in diameter, was present in the thoracic wall

in the 4th intercostal space about 1 cm. to the right of the sternum. A necrotic portion of the 4th rib was exposed in the depths of this sinus but there was no communication with the pleural cavity.

The peritoneal surfaces were smooth and shiny. The cavity contained about 500 cc. of clear yellow fluid. The liver extended 4 cm. below the xiphoid and 3 cm. below the costal margins. The abdominal viscera were grossly normal. The mesenteric lymph nodes were not enlarged. The pleural cavities contained no free fluid. The surfaces of the lungs were smooth and shiny anteriorly, but posteriorly the pleural space on the right was obliterated by firm, fibrous adhesions which also involved the diaphragmatic pleura. An abscess, 2 cm. in diameter, was present in the intercostal muscles of the second interspace just to the right of the sternum. The abscess did not communicate with the skin or pleural cavities but had almost ruptured through the parietal pleura. It was filled with thick, yellow-green pus. The pericardial cavity appeared normal. The heart weighed 200 gm. and appeared to be normal. The lungs together weighed 2,490 gm. Innumerable small, shiny nodules, measuring 2 to 3 mm. in diameter, were visible and palpable beneath the pleura of all surfaces of both lungs. Similar discrete nodules were found throughout the substances of both lungs (Fig. 2). On section, the nodules were firm and yellow without obvious central liquefaction. All lobes were rubbery, subcrepitant, and congested. The hilar and mediastinal lymph nodes were moderately enlarged, firm, and pink. The bronchi were filled with red, frothy fluid. The spleen weighed 220 gm. On section, the parenchyma was reddish purple and studded throughout with firm, yellow nodules resembling those in the lungs. The liver weighed 1,660 gm. Scattered small, yellow nodules were present beneath the capsule but were not found elsewhere in the parenchyma of the liver. The right and left kidneys weighed 176 and 180 gm., respectively. The cortical surfaces were smooth, reddish brown, and were elevated by numerous firm, yellowish nodules similar to those already described. On section, the cortices were similarly studded with firm, yellow nodules measuring up to 8 mm. in diameter. The prostate gland was slightly enlarged and soft. On section an abscess, 0.8 cm. in diameter, was present in the right lateral lobe. Its cavity was filled with thick, yellow pus. The gastrointestinal tract and the remaining viscera were of normal appearance.

The brain weighed 1,330 gm. It was soft and edematous, the gyri were flattened, and the sulci narrowed. The dura mater was not thickened or otherwise abnormal except in a region 3 cm. wide running along the superior sagittal sinus for a distance of 5 cm. in the occipital region. In this region, the under surface of the dura was tenaciously

adherent to a semi-necrotic mass of friable, yellow tissue, 2 to 12 mm. thick, which spread laterally about 3 cm. on either side of the sinus and extended down between the occipital lobes along the falx cerebri to its inferior margin. The lining of the superior sagittal sinus in this region was roughened and granular but the lumen was not occluded. An attempt was made to lift the dura and the adherent necrotic material from the brain surface, but no clear line of demarcation was present. Necrosis extended into the brain to a depth of several mm. over the medial and superior surfaces of the parietal and occipital lobes (Fig. 4). The leptomeninges of the base of the brain, cerebellum, pons, and medulla were covered by a tenacious, thick, yellow-green exudate, while the meninges covering the convexities of the cerebral hemispheres appeared normal. The vessels of the leptomeninges were moderately congested.

### Microscopic Examination

The heart was histologically normal except for slight edema of the pericardium.

The lungs showed numerous uniformly distributed focal areas of necrosis and suppuration, each surrounded by a granulomatous zone. These granulomata varied greatly in size, the larger ones corresponding to the nodules seen grossly. The granulomata occupied about one-half of the area of the sections examined; some were poorly delimited and others were surrounded by a zone of fibrous tissue. They consisted of necrotic débris, polymorphonuclear neutrophils, epithelioid cells, and round or ovoid yeast-like bodies measuring 8 to 12 $\mu$ in diameter. The yeast-like bodies had a highly refractile, doubly contoured cell wall surrounding a granular, central, cytoplasmic mass. Many large multinucleated giant cells were present chiefly at the periphery of the granulomata and some of them contained yeast-like bodies in their cytoplasm (Fig. 3). The alveolar architecture was destroyed within the granulomata but the intervening pulmonary tissue showed remarkably little change except for edema fluid in occasional alveoli.

The spleen contained focal granulomatous and necrotic areas similar to those in the lungs. Some of these lesions contained few, and others many, yeast-like organisms, either free or within giant cells. The intervening splenic pulp was normal except for moderate fibrosis. The malpighian bodies were well preserved and were seldom involved.

The liver showed widely scattered, small, granulomatous lesions containing yeast-like bodies, but was otherwise normal. The gallbladder, pancreas, gastrointestinal tract, and adrenals were normal.

The kidneys contained sharply delimited regions of necrosis, granu-

lomatous inflammation, and suppuration. Many yeast-like organisms were present in these lesions, both free and within the cytoplasm of giant cells. There were degenerative changes of the epithelium of the convoluted tubules and occasional hyaline and granular casts in tubular lumina. The urinary bladder and testes were normal.

The prostate contained multiple abscesses. Many yeast-like organisms were present in the abscess cavities, in the neighboring pus-filled glands, and in the cytoplasm of large multinucleated giant cells.

The mediastinal lymph nodes showed diffuse infiltration with polymorphonuclear neutrophils and marked interstitial fibrosis. Many yeast-like bodies and giant cells were present.

The skin from the lesions on the forehead showed extensive fibrosis of the dermis and subcutaneous tissues. There were many irregular regions of suppuration and granulomatous inflammation surrounded by dense fibrous tissue. Within these regions yeast-like bodies were present, both free and within giant cells.

In the right occipital lobe of the brain and near the midline, the superficial layers of the cortex were destroyed and replaced by granulation tissue and inflammatory exudate. The exudate contained many polymorphonuclear neutrophils together with lymphocytes, plasma cells, and macrophages. Scattered throughout were numerous round, doubly contoured yeast-like bodies, varying in diameter from 6.4 to 12.3 $\mu$. Some of these showed budding (Fig. 11). The cortical tissue beneath the region of superficial destruction showed marked edema and degenerative changes of nerve cells. Gliosis was not apparent. The spaces of the overlying leptomeninges were filled with similar exudate containing many yeast-like organisms. In neighboring regions the meninges contained inflammatory exudate but there was no destruction of the underlying cortex. Sections from several other regions showed no inflammatory changes in the meninges or in the brain.

The cerebellum showed extensive superficial destruction and inflammatory changes of the meninges similar to those described. The cerebral peduncles, pons, and basal ganglia were normal. Extensive inflammatory changes were present in the leptomeninges covering the medulla, and numerous yeast-like bodies were found.

A mass of granulomatous tissue exceeding 1 cm. in thickness was attached to the internal surface of the dura mater in the region of the falx cerebri. Much of this mass was completely necrotic, but in many regions innumerable yeast-like bodies were present, mingled with giant cells, polymorphonuclear neutrophils, macrophages, plasma cells, and lymphocytes. Such collections were in many instances completely surrounded by collagenous fibrous tissue. New blood vessels and fibro-

blasts were present except in regions of necrosis and suppuration. The superior sagittal sinus was almost completely surrounded by tissue of this character and in one region the wall was destroyed. Granulation tissue containing many organisms had replaced the wall of the sinus in this region and had encroached on the lumen.

*Anatomic Diagnosis.* Systemic blastomycosis with involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain.

*Summary of Pathologic Findings.* This case of systemic blastomycosis was characterized by widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating cell. In all of these lesions there were numerous round, oval, yeast-like bodies, either free or within giant cells, measuring 4.6 to 14.0 $\mu$ in diameter, which resembled *B. dermatitidis*. Lesions were found in the lungs, liver, spleen, and kidneys. In addition, there were cutaneous lesions of the face which lacked the typical gross appearance of blastomycotic ulcers but nevertheless contained organisms. Subcutaneous abscesses and lesions of the ribs, brain, and meninges were present also. Grossly and microscopically, the lesions in the lungs bore some resemblance to miliary tubercles, but were more like miliary abscesses in that the granulomatous zones were heavily infiltrated with polymorphonuclear neutrophils and their centers were suppurative rather than caseous. The histologic characteristics of the lesions in this case were similar to those described by Baker.[3]

## Primary Isolation and Identification of the Fungus

The method of primary isolation of the fungus was similar to those recommended by Benham[6] and Martin and Smith.[7] Direct examination of exudates was made by means of unstained moist mounts, in saline solution and in 10 to 20 per cent NaOH, and by Wright's staining. Numerous spherical, doubly contoured, thick-walled, yeast-like cells were seen. Exudate was streaked by the gridiron technic on agar plates containing 5 per cent sheep's blood with heart infusion base and Sabouraud's dextrose agar (pH 5.6). Incubation was at 37° C. and room temperature, respectively. Growth on Sabouraud's agar, at room temperature, was not apparent until 7 days after inoculation, when many small colonies of a white, filamentous mold appeared along the lines of streaking.

*Growth at 37° C.* Growth appeared on blood agar on the fourth day of incubation in the form of numerous well isolated, tiny, filamentous, fungus colonies growing along the lines of streaking. Transfer of one colony from this plate to a blood agar slant resulted in the development

4 days later of a mealy, adherent growth resembling the growth of *Mycobacterium tuberculosis* on glycerin media. A giant colony of this type is shown in Figure 5. Microscopic examination of the growth revealed numerous large, round, thick-walled, doubly contoured, single or budding cells, 5.7 to 16.4 $\mu$ in diameter, and thin, segmented mycelial strands (Fig. 7). Upon second and third serial transfers to blood agar slant and Sabouraud's agar, the culture underwent characteristic gross and microscopic changes with complete suppression of mycelium formation and development of only large budding cells. The progression of changes in the microscopic appearance of the culture is illustrated in Figures 7, 8, and 9. The third serial transfer of the organism produced a mealy, wrinkled, cerebriform and friable growth of fungus which possessed no cottony, aerial mycelium. It was noted that frequent transfers favored the mealy type of growth.

*Growth Characteristics at Room Temperature.* A single transfer of the organism growing at 37° C. to either Sabouraud's or blood agar at room temperature caused the prompt reversion of structure, with development of an abundant wooly, white, aerial mycelium (Fig. 6). Further study of the organism growing in a Henrici-type slide culture [8] at room temperature revealed the development of abundant aerial mycelium and numerous oval to round conidia, 3 to 4 $\mu$ in diameter, which were attached to hyphae near the segmentations. Other round to pear-shaped conidia of the same size developed on lateral sterigmata of varying lengths (Fig. 10). Submerged mycelium developed raquette hyphae and thick, swollen structures resembling intercalary chlamydospores, 7.5 to 18 $\mu$ in diameter.

*Growth Characteristics on Carrot Slant.* At 37° C., the organism produced well isolated, warty, wrinkled, cerebriform colonies similar to those produced on blood agar, but considerably more raised. Ascospore formation was not observed at any time. The new strain of fungus appeared to be identical in all its characteristics with a stock laboratory strain of *Blastomyces dermatitidis.**

*Cultural Variation at 37° C.* After several subcultures on blood agar and prolonged incubation at 37° C., minute prickly elevations consisting of closely packed filaments of true mycelium appeared on the surface of the growth. These were considered to be abortive coremia representing an attempt of the fungus to revert to the myceliated state. Both the stock and newly isolated strains of *B. dermatitidis* exhibited this characteristic, and also showed the other two cultural variations described for the fungus by Henrici.[8] These forms were: (1) mealy colony, unicellular form at 37° C. (Figs. 5, 7, 8, and 9); (2) prickly,

* Furnished by Dr. Norman F. Conant, Duke University, Durham, N.C.

abortive coremia at 37° C.; and (3) wooly, mycelial form at room temperature (Figs. 6 and 10). In summary, it may be said that the morphologic appearance of *B. dermatitidis* on agar medium is variable, depending on temperature of incubation, age of the culture, and number and rapidity of transfers from the original isolation. Recent studies by Levine and Ordal [9] on factors influencing the structure of *B. dermatitidis* on peptone glucose medium showed that the wooly, mycelial form was predominant in cultures growing from "room temperature" through 33° C., and the mealy, yeast-like form from 35° C. through 37° C.

*Biochemical Reactions.* Both the newly isolated strain of *B. dermatitidis* and the stock laboratory strain, which had been maintained in the budding cell stage at 37° C., were inoculated into beef infusion broth, pH 6.8, containing 1 per cent of one of the following sugars: maltose, lactose, sucrose, and dextrose. At 37° C., both strains developed abundant, colorless, submerged mycelium in the liquid media rather than the fragmented, mealy type of growth of the organism on solid media at 37° C. Cultures were maintained for 6 weeks, during which time growth of the fungus filled the media, but failed to produce any discernible gas or change in reaction. This does not preclude, however, the utilization of any of the carbohydrates by either strain of Blastomyces.

## In Vitro Resistance of Blastomyces Dermatitidis to Streptomycin and Penicillin

Little information is available concerning the effect of the antibiotics, streptomycin and penicillin, on *B. dermatitidis*. Since the accepted method of treatment of North American blastomycosis with iodides and x-ray therapy is not particularly effective, the *in vitro* action of streptomycin and penicillin against the organism was thought worthy of examination.

Series of tubes of Sabouraud's dextrose broth and nutrient broth containing quantities of streptomycin hydrochloride (Merck) and sodium penicillin (Wyeth) varying from 0.1 to 200 units per cc. were inoculated with standard laboratory strains of *Staphylococcus aureus* and *Escherichia coli* as control organisms, and with two strains of *Blastomyces dermatitidis,* the newly isolated and the laboratory stock culture. The fungus inoculum consisted of 6-day-old budding cells, maintained in the unicellular state on blood agar at 37° C. Incubation was at 37° C. and observations were made at suitable intervals. The two strains of the fungus were found resistant to concentrations of streptomycin and penicillin up to 200 units per cc., as illustrated in

Table I. In order to verify these results, the "window" method of Cooke,[10] originally devised for assay of penicillin and for the determination of penicillin sensitivity of bacteria, was used to determine the penicillin and streptomycin sensitivity of *B. dermatitidis*. Blood agar was used as the culture medium. Both strains of Blastomyces again were found to be insensitive to concentrations up to 200 units per cc.

TABLE I

*In Vitro Resistance of Blastomyces dermatitidis to Streptomycin and Penicillin*

| Concentration | Growth in Sabouraud's dextrose broth | | | |
|---|---|---|---|---|
| Units per cc. | *Blastomyces dermatitidis** | *Blastomyces dermatitidis* (Conant) | *Staphylococcus aureus*† | *Escherichia coli*† |
| Streptomycin hydrochloride | | | | |
| 0 | + | + | + | + |
| 0.1 | + | + | + | + |
| 0.5 | + | + | + | + |
| 1.0 | + | + | + | + |
| 5 | + | + | + | − |
| 10 | + | + | ± | − |
| 20 | + | + | − | − |
| 50 | + | + | − | − |
| 100 | + | + | − | − |
| 200 | + | + | − | − |
| Sodium penicillin | | | | |
| 0 | + | + | + | |
| 0.1 | + | + | + | |
| 0.5 | + | + | − | |
| 1.0 | + | + | − | |
| 5 | + | + | − | |
| 10 | + | + | − | |
| 20 | + | + | − | |
| 50 | + | + | − | |
| 100 | + | + | − | |
| 200 | + | + | − | |

* Isolated from case of fatal North American blastomycosis.
† Same results in nutrient broth.

of either antibiotic. The fact that penicillin did not affect the growth of two strains of *B. dermatitidis* when cultured at 37° C. on liquid or solid medium is in keeping with the clinical failure of penicillin therapy in a case of North American blastomycosis reported by Herrell, Nichols, and Heilman.[11] The results obtained by us are in agreement with the recent observations of Meyer and Ordal [12] who reported that streptomycin and penicillin were not fungistatic to one strain of *B. dermatitidis*.

## DISCUSSION

### *Demonstration of Fungal Elements in Exudates*

Pus and exudates are cleared by placing them in a drop of 10 to 20 per cent sodium hydroxide (or potassium hydroxide), mounting a

coverslip on the preparation and gently warming the slide. Since numerous artifacts are easily confused with round or oblong fungal cells, a vaseline-rimmed mount of the fresh untreated specimen may be kept at room temperature for 24 to 48 hours and examined for either budding cells or development of hyphal germ tubes and hyphal elements from the suspected fungal spores.

Routine Gram staining of smears for demonstration of fungal elements is unsatisfactory because of the distorting effect of heat fixation. Gram stains of alcohol-fixed smears or direct Wright stains generally yield better results. Direct examination of unstained exudates in our case of blastomycosis disclosed the presence of numerous nonbudding, oval, thick-walled, refractile bodies, which, when maintained in a moist, untreated mount at room temperature for 24 hours, formed single, small buds compatible in structural characteristics with cells of *B. dermatitidis*. The wall of the oval cell was thick, highly refractile, and appeared as a bright band bordered by two thin, dark lines, which imparted a doubly contoured appearance to the unstained cell. The cell wall of the single bud, on the other hand, was considerably thinner than that of the parent cell.

Although the finding of budding, thick-walled, doubly contoured cells is suggestive of Blastomyces, it should be stressed that the doubly contoured appearance is by no means confined to this genus. Budding cells of certain species of Torula and Candida possess double contours which may be even more prominent than those of Blastomyces. Immature spherules of *Coccidioides immitis,* not possessing endospores, appear doubly contoured and resemble nonbudding forms of *Blastomyces dermatitidis* and *Paracoccidioides* (*Blastomyces*) *brasiliensis.* Furthermore, Cryptococcus cells possess rather thick cell walls and present double contours, as well as the spherical or oblong cells of Geotrichum. The discovery of budding, thick-walled, doubly contoured cells in exudates, although highly suggestive of *Blastomyces dermatitidis,* should, therefore, not be considered as ultimate proof of diagnosis of North American blastomycosis. As aptly phrased by Martin and Smith: [1]

"The diagnosis can be proved unequivocably only by the isolation and identification of the infecting organism, *Blastomyces dermatitidis.* However, from the history and clinical appearance of the infection, direct examination of material from the lesions, skin tests, and complement fixation reactions, tentative diagnoses can be made which should stimulate efforts toward isolation of the fungus."

Baker [3] believed that the tissue reaction produced by *B. dermatitidis* is sufficiently distinctive to permit histopathologic diagnosis with a high degree of accuracy if Blastomyces-like organisms are present in the lesion, but attempts should be made to obtain pure cultures in every case.

TABLE II

*Microscopic Features of Oval, Spherical, or Oblong Fungal Cells More or Less Resembling Yeasts and Capable of Producing Granulomatous Lesions in Tissues*

| Disease and organism | In exudates[2,6,13] | In stained tissue section[2,6,13] | Measured size in culture and tissue section | | | | | | Literature | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cell type or shape | Age | Temperature of incubation | Medium | Width x length: Smallest size | Width x length: Largest size | Source | Size | Reference no. |
| | | | | *days* | | | μ | μ | | μ | |
| North American Blastomycosis | | | | | | | | | Culture# | 7–18 | 13 |
| | | | | | | | | | Exudate | 3–24 | 8 |
| | | | | | | | | | Exudate | 5–20 | 14 |
| *Blastomyces dermatitidis** | Spherical, thick-walled, yeast-like organisms appearing doubly contoured and forming single buds. | Similar to appearance in exudates. Double contour more prominent due to shrinking of cytoplasm. Cytoplasm stains with variable intensity. | Oval | 5 | 37°C. | Blood agar | 4.6 x 5.7 | 12.8 x 16.4 | Exudate | 7–18 | 13 |
| | | | | | | | | | Exudate | 8–15 | 2 |
| | | | | | | Tissue section | 4.6 x 6.6 | 12.3 x 14.0 | Tissue | 3–24 | 8 |
| | | | | | | | | | Tissue | 5–20 | 14 |
| | | | | | | | | | Tissue | 8–20 | 6 |
| South American Blastomycosis | | | | | | | | | Culture# | 3–25 | 13 |
| | | | | | | | | | Exudate | 10–60 | 2 |
| | | | | | | | | | Exudate | 10–69 | 8 |
| *Paracoccidioides (Blastomyces) brasiliensis*† | Spherical, thick-walled, yeast-like organisms forming multiple buds. | Some forms are indistinguishable from *B. dermatitidis;* others show minute, dark staining projections about periphery of parent cell which represent multiple buds. Minute buds found free and within cytoplasm of giant cells. Large, multiple peripheral buds from parent cell also seen. | Parent cell | 10 | 37°C. | SAB¶ | 8.2 x 8.2 | 18.9 x 18.9 | Exudate | 1–30 | 14 |
| | | | | | | | | | Tissue | 10–60 | 17 |
| | | | | | | | | | Tissue | 1–30 | 14 |
| | | | Single bud | 10 | 37°C. | SAB | 4.6 x 4.6 | 9.1 x 9.1 | Exudate | 10–30 | 2 |
| | | | Tuberculate buds of parent cell | 10 | 37°C. | SAB | 1.6 x 1.6 | 4.6 x 4.6 | Exudate | 1–10 | 2 |
| Geotrichosis | | | | | | | | | | | |
| *Geotrichum sp.*† | Spherical, rectangular, or oblong, thick walled cells. May be confused with *B. dermatitidis.* | Not reported. | Oval | 6 | Room | SAB | 5.7 x 7.4 | 8.2 x 10.7 | Culture# | 4–12 | 2 |
| | | | Oval | 40 | Room | SAB | 4.9 x 8.2 | 10.7 x 10.7 | Exudate | 4–10 | 2 |
| | | | Oblong | 6 | Room | SAB | 5.7 x 8.2 | 6.6 x 16.4 | Exudate | 4–8 | 2 |
| | | | Oblong | 40 | Room | SAB | 4.9 x 8.2 | 14.6 x 20.5 | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cryptococcosis | | | | | | | | | Culture# | 4–5 | 6 |
| | | | | | | | | | Exudate | 5–20 | 2 |
| *Cryptococcus neoformans*† | Oval to spherical, forming single buds, thick-walled, yeast-like organisms; wide refractile capsule demonstrable in India ink preparation. | Oval to spherical organisms surrounded by irregular, nonstaining, mucinous capsule; single buds; best seen in Gram-stained sections. | Oval | 5 | Room | SAB | 2.5 x 2.5 | 6.6 x 8.2 | Exudate | 5–10 | 14 |
| | | | | | | | | | Exudate | 2–15 | 8 |
| | | | Oval | 35 | Room | SAB | 2.5 x 2.5 | 6.6 x 8.2 | Tissue | 5–15 | 6 |
| | | | | | | | | | Tissue | 5–10 | 14 |
| Moniliasis | | | | | | | | | | | |
| *Candida albicans*† | Small, oval, thin-walled, yeast-like organisms, forming single buds. | May be confused with lymphocytes in preparations stained with hematoxylin and eosin, but not in Gram-stained sections; cell stains darkly with Gram stain but capsule stains poorly. Both oval cells and mycelial elements are present. Histopathologic diagnosis difficult without culture. | Oval | 5 | Room | SAB | 3.3 x 3.3 | 8.2 x 8.2 | Culture# | 3–6 | 18 |
| | | | | | | | | | Exudate | 2.5–4 | 2 |
| | | | Oval | 40 | Room | SAB | 2.5 x 2.5 | 9.0 x 9.0 | Tissue | 4 | 2 |
| Coccidioidomycosis | | | | | | | | | Exudate | 2–80 | 14 |
| | | | | | | | | | Exudate | 20–80 | 2 |
| *Coccidioides immitis*† | Nonbudding, thick-walled spherules filled with numerous endospores which are released in tissues and which increase in size with maturity. Immature spherules not containing endospores are doubly contoured and resemble nonbudding forms of *B. dermatitidis* and *P. brasiliensis*. | Many forms indistinguishable from nonbudding forms of *B. dermatitidis* and *P. brasiliensis*; heavy doubly contoured walls; material within cell stains irregularly, darker just adjacent to capsule than in center of cell. Absence of budding and presence of endospores are diagnostic. | Spherule‡ | | | Tissue section | 12.8 x 16.4 | 46.0 x 46.0 | Exudate | 5–50 | 8 |
| | | | | | | | | | Tissue | 2–80 | 14 |
| | | | | | | | | | Tissue | 2–60 | 15 |
| | | | | | | | | | Tissue | 20–80 | 8 |
| | | | | | | | | | Tissue | 6–50 | 6 |
| | | | Endospore‡ | | | Tissue section | 2.5 x 2.5 | 5.7 x 5.7 | Exudate | 2–5 | 2 |
| | | | | | | | | | Tissue | 1–4 | 8 |

TABLE II (Continued)

| Disease and organism | In exudates[2,8,13] | In stained tissue section[2,8,13] | Measured size in culture and tissue section | | | | | | Literature | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cell type or shape | Age | Temperature of incubation | Medium | Width x length: Smallest size | Width x length: Largest size | Source | Size | Reference no. |
| | | | | *days* | | | *μ* | *μ* | | *μ* | |
| Chromoblastomycosis<br>*Hormodendrum compactum*†<br>*Hormodendrum pedrosoi*†<br>*Phialophora verrucosa*† | All three forms are single or clustered, round, thick-walled, dark brown bodies; multiply by splitting and not by budding. | All three forms identical; brown, round, clustered, septate cells. | Oval conidia | 14 | Room | SAB | 1.6 x 1.6 | 2.0 x 2.0 | Culture # | 1.5–3.0 | 16 |
| | | | Oval conidia§ | 14 | Room | SAB | 1.6 x 1.6 | 1.6 x 2.5 | Culture #<br>Culture # | 1.5-2.5§<br>1.5-6.0△ | 16<br>2 |
| | | | Oval conidia | 14 | Room | SAB | 1.6 x 1.6 | 2.5 x 4.6 | Culture # | 1.5-4.0 | 16 |
| Rhinosporidiosis<br>*Rhinosporidium seeberi* | Round to oval spores possessing thin cell walls and containing several granules of uniform size. Spores mature to large sporangia. | Numerous large sporangia which are empty or filled with many oval, thin-walled spores; the latter may also be found free in tissues. | Sporangia‖ | | | Tissue section | 21 x 21 | 177 x 206 | Tissue | 50-300 | 17 |
| | | | Spores‖ | | | Tissue section | 3 x 3.8 | 12 x 12 | Tissue | 8–9 | 17 |

* Isolated from a case of fatal systemic blastomycosis.
† Furnished by Dr. N. F. Conant, Duke University, Durham, N.C.
‡ One tissue section.
§ Phialophora type of conidia.
‖ Nasal mucosa, one case.
¶ Sabouraud's agar.
# Age of culture not reported.
△ Hormodendrum type of conidia.

In support of these statements, we might point to the wide variety of fungi, as listed in Table II, which appear in exudates as oval, spherical, or oblong, refractile, fungal cells more or less resembling yeasts and capable of causing granulomatous lesions in tissues. In Table II there are also presented careful measurements of fungal cells and spores made at different ages of culture by one of us (M.L.L.) and compared with sizes reported in the literature. In this study, measurements were made of only one representative culture. Strain variation was not investigated. In one study of strain variation of Microsporum by Conant,[19] spore dimensions were found to be too variable for species differentiation. The wide variation in dimensions between the smallest and largest spore in a given culture of fungus and between strains of the same species is presumably dependent upon many factors of growth and development, none of which were studied here. Differences in spore size among the several genera of fungi presented in Table II are sufficient, however, to permit rough identification of the fungi in human exudates and stained tissues. Cultures should be attempted in every case in which a fungus disease is suspected.

### *Technic of Primary Isolation*

Media suitable for *B. dermatitidis,* as well as for other fungi, are Sabouraud's dextrose or maltose agar incubated at room temperature, and blood agar incubated at 37° C. Specimens are streaked by the gridiron technic on both media in such a manner as to yield isolated fungal colonies. Growth of pathogenic fungi on primary isolation will appear in most cases within 7 to 10 days on Sabouraud's dextrose agar at room temperature, but may sometimes require as long as 16 days. Many air-borne nonpathogenic fungi, which are laboratory contaminants, on the other hand, produce abundant, rapidly spreading aerial mycelium in as short a time as 48 to 72 hours and may overgrow the entire plate. Care must be taken to minimize contamination by air-borne fungal spores during the pouring, storage, and inoculation of the media.* The use of a small, closed room, rendered relatively dust-free and kept shut, will increase the proportion of successful primary isolations. Once a pure culture of a fungus is obtained, it is easier to maintain its purity on Sabouraud's agar slant than on an agar plate because of the reduced exposure to air-borne fungal spores. As soon as visible, filamentous, mold-like colonies develop in the agar along

* A new agar medium for primary isolation of fungi, obviating many of the difficulties due to air-borne contamination and inhibiting bacteria, recently has been developed by the senior author and is now undergoing clinical trial.

An account of this medium has been published since this manuscript was submitted. Littman, M. L., A culture medium for the primary isolation of fungi. *Science,* 1947, **106,** 109–111.

the lines of streaking, one entire small colony is dug out with a platinum loop and transferred to a Sabouraud's agar slant. Although the young colony will usually not have developed spores, the vegetative mycelium is nevertheless quite capable of further growth and subsequent sporulation in the transplant.

All fungi incubating at room temperature are kept in a darkened cabinet since direct sunlight may inhibit growth. Streaking of a blood agar plate with fresh exudate and incubation at 37° C., as recommended by Martin and Smith,[7] and Conant *et al.*,[2] will increase the likelihood of successful primary isolation. When the fungus is obtained in the pure state, free of bacteria and other fungi, attempts at identification may be continued by the slide culture technic, giant colony study, identification of characteristic sporulating elements in special media, and studies of animal pathogenicity and tissue reactions. If suitable facilities are not available locally, a pure culture may be sent to a specialized laboratory for identification.

Primary isolation and subsequent identification of pathogenic fungi from lesions in patients usually is not a difficult task for an experienced mycologist. It does present real difficulties to the bacteriologist or pathologist who is unfamiliar with the special methods and media required. Demonstration and primary isolation of certain fungi from mycotic lesions are simple if certain basic procedures are followed in the treatment of the exudate:

*Direct Examination.* (1) Mount in 10 per cent NaOH; (2) Gram stain or Wright stain; (3) incubation of unstained saline mount to detect sprouting or budding; (4) rough identification of the fungus with the help of Table II (spherical or oval fungal cells only).

*Culture.* (1) Streaking on Sabouraud's and blood agar plates, and incubation at room temperature and 37° C., respectively; (2) early transfer of young mold colony to Sabouraud's agar slant; (3) slide culture; (4) giant colony study; (5) sporulating media; (6) animal pathogenicity; (7) animal tissue reactions.

## Summary and Conclusions

A case of fatal North American blastomycosis presented, on autopsy, involvement of the skin, subcutaneous tissues, lungs, liver, spleen, kidneys, prostate, bone, meninges, superior sagittal sinus, and brain. Histopathologic examination showed widespread abscesses and granulomatous foci in which the polymorphonuclear neutrophil was the predominating inflammatory cell.

Cultures isolated from exudates and from organs obtained at autopsy were identified as *Blastomyces dermatitidis*. Budding cell forms of the

fungus were found to vary in size in culture from 4.6 by 5.7 $\mu$ (width and length) to 12.8 by 16.4 $\mu$, and in various organs of the body from 4.6 by 6.6 $\mu$ to 12.3 by 14.0 $\mu$.

Two strains of *B. dermatitidis* were found to be resistant to streptomycin and penicillin in concentrations up to 200 units per cc. For this reason it is not likely that either of these antibiotics will prove effective in the clinical treatment of North American blastomycosis.

Acknowledgments are made to Drs. Charles E. Dunlap, Guillermo M. Carrera, and Morris F. Shaffer of the Department of Pathology and Bacteriology, Dr. Roy H. Turner of the Department of Medicine, School of Medicine, Tulane University of Louisiana, and Dr. Emma S. Moss of the Department of Pathology, Charity Hospital, New Orleans, for their advice and guidance in the preparation of the manuscript.

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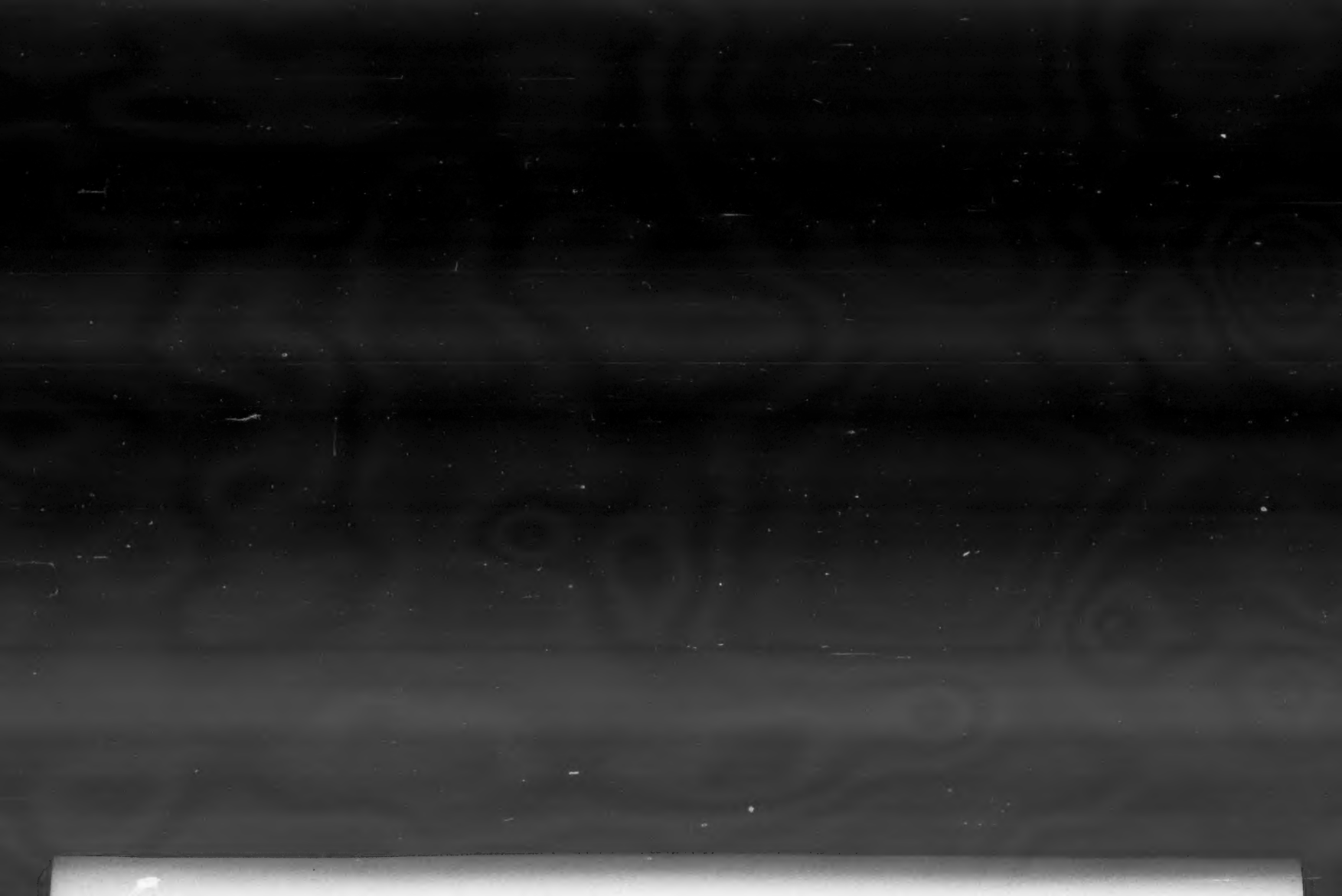
## DESCRIPTION OF PLATES

### PLATE 66

FIG. 1. Roentgenogram of the chest showing diffuse bilateral, miliary, pulmonary lesions in North American blastomycosis. Heart and mediastinal structures are within normal limits. The picture is compatible with miliary tuberculosis.

FIG. 2. Lung. The surface made by cutting shows firm, yellow nodules with no apparent central liquefaction.



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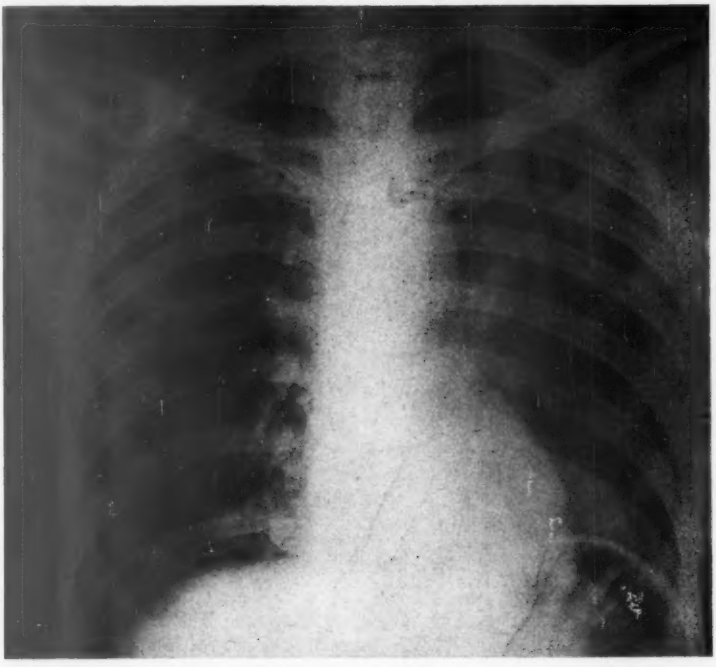

2

PLATE 67

FIG. 3. Lung. Photomicrograph of the periphery of a focal granuloma showing epithelioid cells and giant cells containing thick-walled, doubly contoured yeast-like bodies of *B. dermatitidis*. × 1000.

FIG. 4. Brain. Superficial necrosis of the cortex of the parietal-occipital lobes near the midline. Of note are the thickening of the dura mater and the presence of a granulomatous exudate attached to the inner surface of the falx cerebri.

3

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3

4

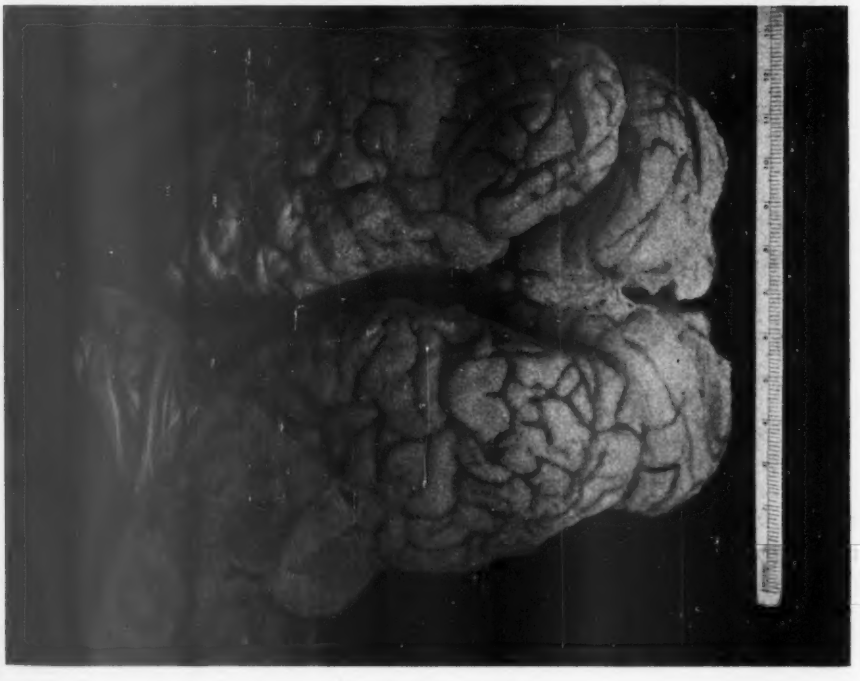

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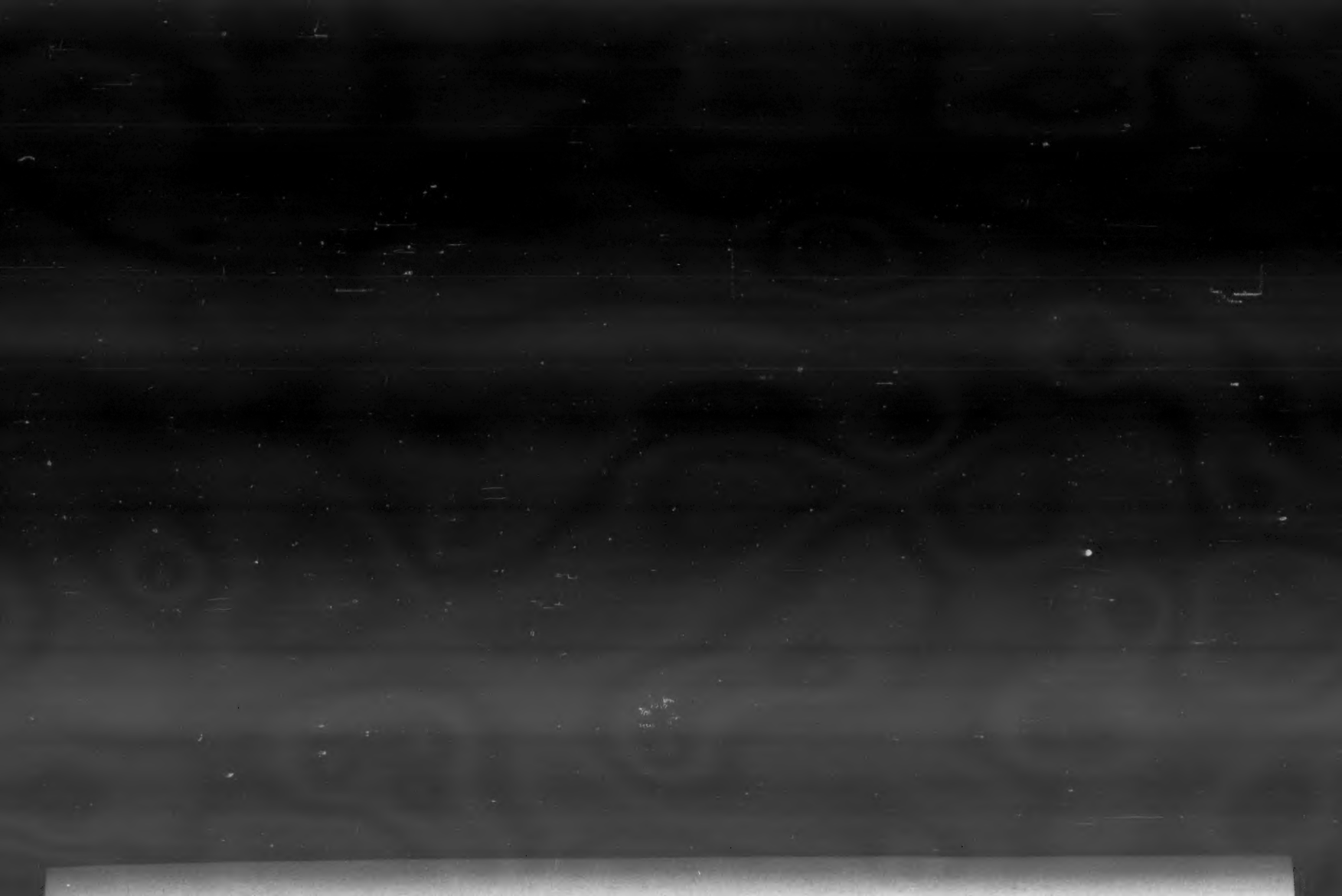
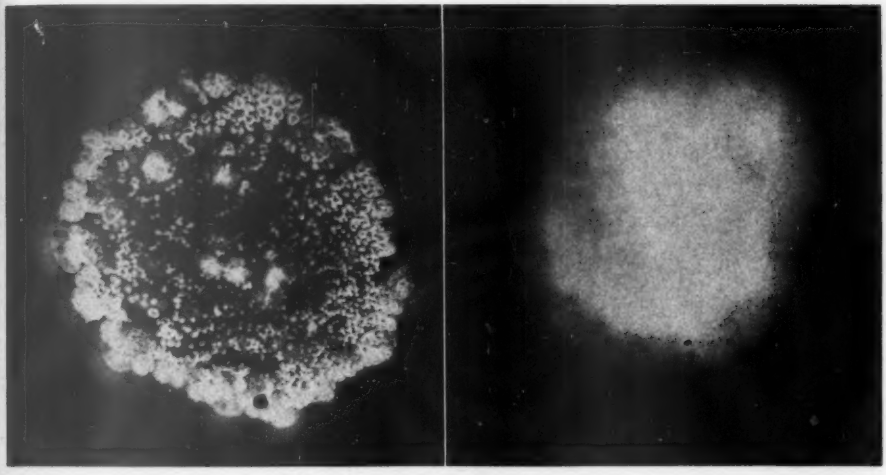
PLATE 68

FIG. 5. Giant colony of *B. dermatitidis,* incubated at 37° C. on Sabouraud's dextrose agar, showing typical mealy, wrinkled, cerebriform growth. The light gray periphery of the colony represents that portion of the growth which has penetrated into the agar. First transfer of newly isolated mold from growth at room temperature. (Mealy colony, original size.)

FIG. 6. Giant colony of *B. dermatitidis* incubated at room temperature on Sabouraud's dextrose agar, showing wooly mycelial appearance. (Wooly mycelial colony, original size.)

FIG. 7. Microscopic appearance of giant colony grown at 37° C., as seen in Figure 5. There are numerous round, doubly contoured, single or budding cells as well as numerous fragmented mycelial strands. First transfer from wooly growth at room temperature. (Mealy state.) × 1000.





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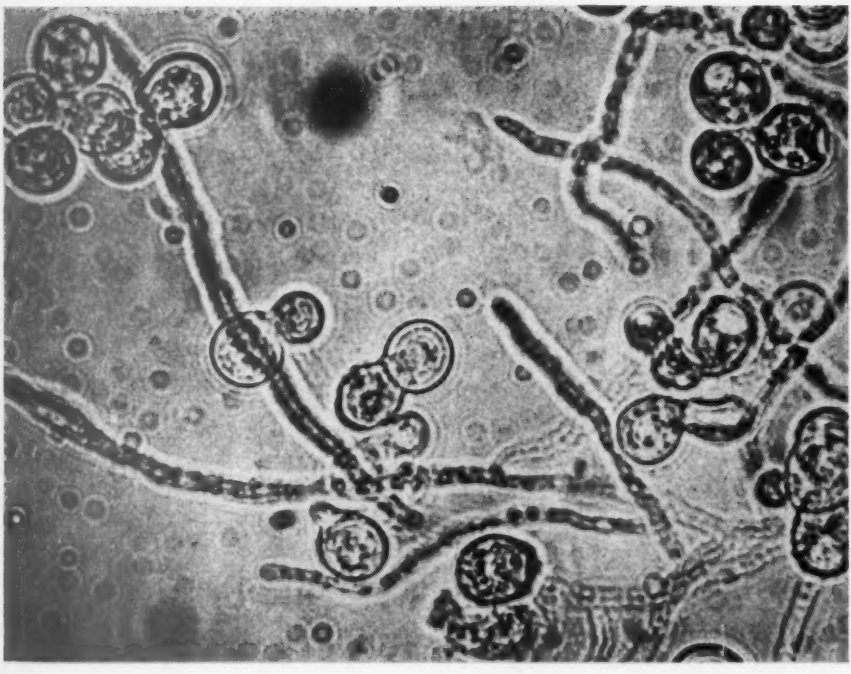

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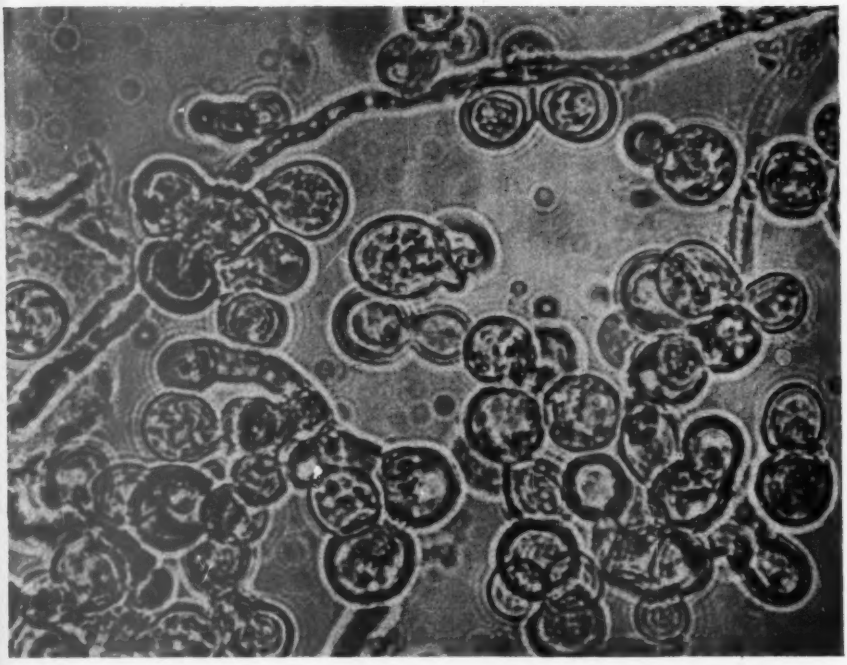
PLATE 69

FIG. 8. *B. dermatitidis*, second transfer, growth at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and relatively few mycelial strands. Age, 5 days. (Mealy state.) × 1000.

FIG. 9. *B. dermatitidis*, third transfer, growing at 37° C. on Sabouraud's dextrose agar. There are numerous budding cells and very few mycelial strands. Age, 5 days. (Mealy state.) × 1000.



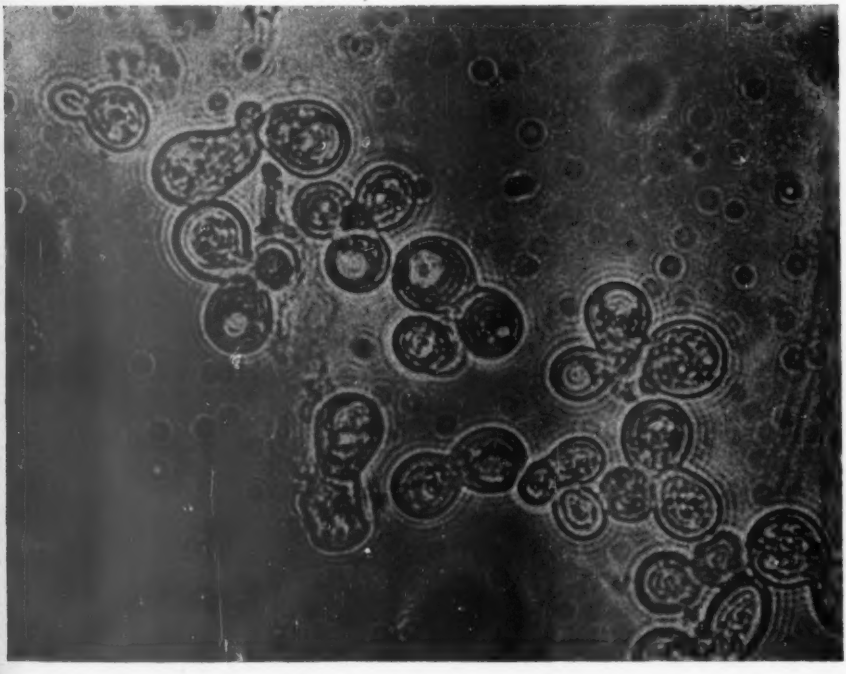
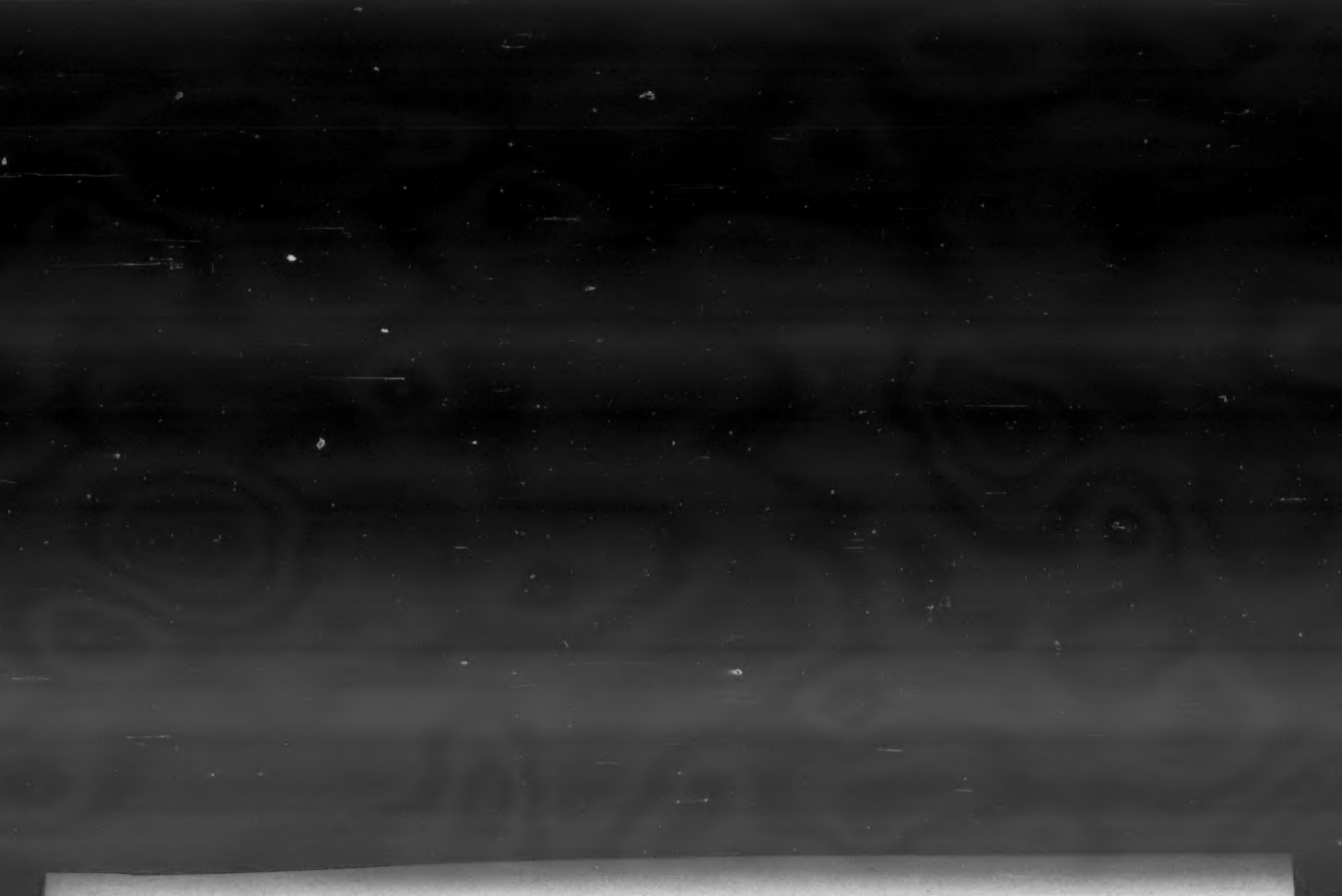
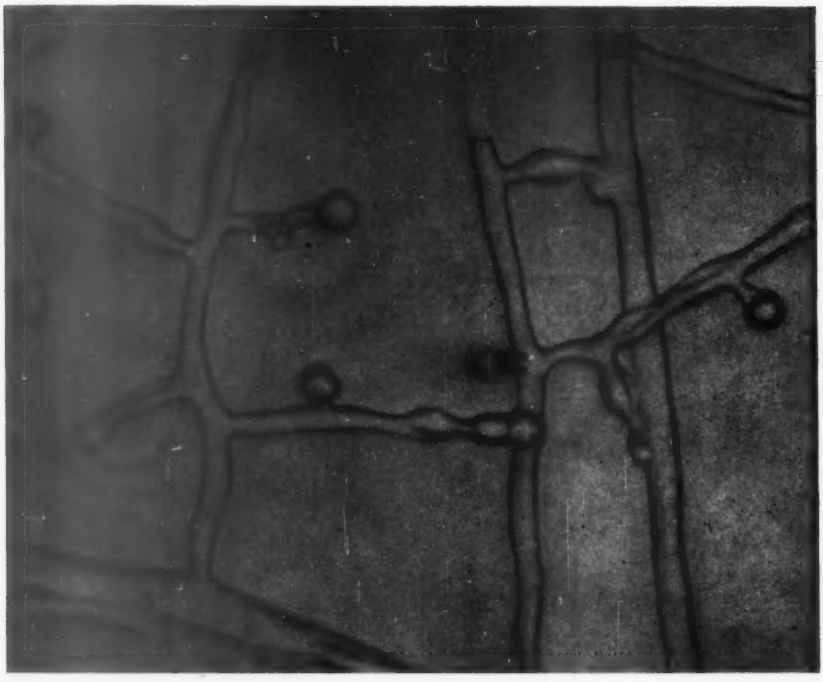


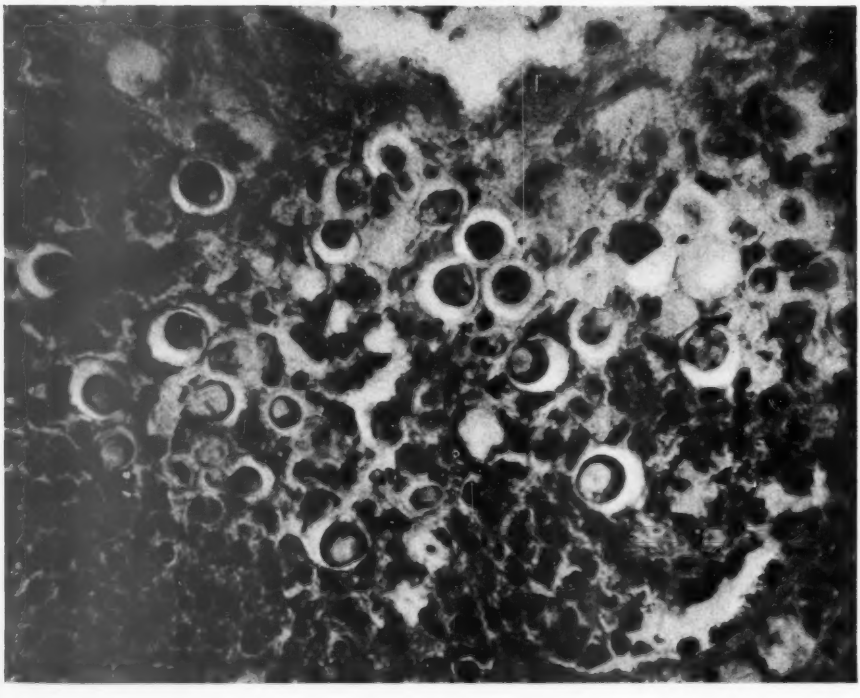
PLATE 70

FIG. 10. Aerial mycelium of *B. dermatitidis* growing in a Henrici-type slide culture at room temperature. Oval, sessile conidia and other oval to pear-shaped conidia are attached to lateral sterigmata. Age, 14 days. (Wooly mycelial state.) × 1200.

FIG. 11. Brain. Photomicrograph of involved portion of the right occipital lobe, showing numerous thick-walled budding cells, varying in diameter from 6.4 to 12.3 μ. Cytoplasmic staining of the fungal cells varies in intensity. × 1000.







# PERSISTENT "INSECT BITES" (DERMAL EOSINOPHILIC GRANULOMAS) SIMULATING LYMPHOBLASTOMAS, HISTIOCYTOSES, AND SQUAMOUS CELL CARCINOMAS *

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In 1942, opportunity was afforded at the Army Institute of Pathology to review the histologic slides of a lesion said to have been produced by a tick bite. The microscopic sections seemed at the time indistinguishable from mycosis fungoides or Hodgkin's disease, especially in view of the presence of multiple lesions in the patient. However, following the study of the cutaneous reactions to arthropods (ticks, mosquitoes, and chiggers), it was quickly appreciated that not only were these diagnoses of neoplasia wrong but that the misinterpretation of these reactions was a common and serious error.[1] The errors involved the misconstruction not only of the dermal reaction but also of the epidermal changes. The latter response was confused with squamous cell carcinoma; the dermal infiltrate was mistaken for mycosis fungoides, Hodgkin's disease, lymphosarcoma, giant follicular lymphoblastoma, and Spiegler-Fendt sarcoid. Undoubtedly the principal reason for the failure to attribute these reactions properly to bites of arachnida and insects was referable to the general impression, despite clear-cut clinical histories, that such reactions last only for days, whereas, in truth, they may persist for as long as 2 years. More recently, the problem has been further complicated by introduction into the literature of a lesion called "eosinophilic granuloma of skin," an entity of questionable nosologic justification.[2-4] Therefore, because of the major importance of establishing a definitive diagnosis and because of the interest in the pathogenesis of a much mimicked histologic pattern, it was felt of use to record the experience in this matter.

## MATERIAL

There were available for study the histologic preparations of reactions to "bites" of 20 arthropods, including 9 ticks, 4 mosquitoes, 3 chiggers ‡ ("red bugs"), and 4 insects not otherwise identified. For comparison there were available the sections of 30 eschars of scrub typhus (tsutsugamushi disease) and 2 sections of skin infected with the ovum or larva of worms. The eschars produced by mites of the

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‡ The "chiggers" are the larval mites of *Trombicula irritans* and are to be distinguished from "jiggers," or adult sand fleas (*Pulex penetrans*).

species Trombicula offered no diagnostic problems inasmuch as they were all from active cases of scrub typhus. No examples of the late residue of such eschars were studied.

The lesions occurred on the extremities in 8 cases, on the abdomen in 5, on the back in 3, on the penis in 2, and in the axilla in 2. Their duration varied from 3 weeks to approximately 2 years; 6 were present longer than 1 year. All but one of the patients were white; in the one Negro, the lesion, following a tick bite, lasted 15 months before it was excised. Two of the group were females. The ages ranged from 21 to 49 years. In 3 instances, the ages were not recorded. Twelve of the 20 bites were acquired in California, Georgia, Texas, and Louisiana. The clinical appearance of the lesions varied from a patch of eczema such as might be caused by an irritant of dermatitis venenata, to a smooth, firm, erythematous, persistent, pruritic, slowly growing or stationary papule. The latter form of lesion simulated a fibroma. Some of the lesions were excoriated or ulcerated; some oozed, formed crusts, and desquamated. One of the lesions on the penis suggested a syphilitic chancre. There was a tendency for the ulcerations to heal, to recur subsequently, and to heal again. In such lesions a portion of the tick was left embedded in the skin. Marked pruritis was a particularly prominent and constant symptom. The lesions varied from 1 to 7.5 cm. in maximum diameter. It was generally impossible to estimate the duration from their clinical appearance.

## Findings

The histologic picture as stated had two distinct components which might or might not be combined: (1) changes in the epidermis and (2) the dermal infiltrate.

### *Epidermal Reaction*

The most striking feature of the epidermal reaction was the marked pseudo-epitheliomatous hyperplasia which occurred in 7 of the 20 instances (2 chiggers, 2 ticks, and 3 unidentified). In 6 of the 7 cases, lesions with the epidermal hyperplasia were submitted to the Institute with the incorrect diagnosis of squamous cell carcinoma (Figs. 1 to 4). The pseudo-epitheliomatous hyperplasia was quite like that often seen at the margins of cutaneous ulcers. The differentiation of pseudo-epitheliomatous hyperplasia from carcinoma was by no means always a simple matter inasmuch as a disruption of the epidermal basement membrane and an excessive number of mitotic figures in the advanced portions of the rete pegs were occasionally present (Fig. 4). The diagnosis of hyperplasia rested on the conclusion that the isolated epi-

dermal nests were not truly invasive but were obliquely cut pegs actually continuous with the epidermis. Moreover, if the cells in mitosis were excepted, the other cells in these deep nests were essentially as differentiated as those of the neighboring uninvolved epidermis. The disruption of the basement membrane was regarded as the effect of the adjacent extensive, compact, inflammatory reaction. In this connection it was noted that pseudo-epitheliomatous hyperplasia was in each instance accompanied by marked dermal inflammation and was most pronounced over the densest portion of the dermal reaction.

Other changes in the epidermis included varying degrees of spongiosis (Fig. 6) which in 4 lesions (2 ticks, 1 chigger, 1 unidentified) reached the degree of vesiculation (Figs. 3 and 5). The vesicles were multiloculated, eczematous collections of serum containing purulent exudate or, as occurred in one instance, masses of eosinophilic leukocytes. The roof of the vesicles and the adjacent epidermis tended to be parakeratotic. Often loosely scattered neutrophilic and a few eosinophilic leukocytes were located in the epidermis, particularly in the spongiotic areas (Fig. 6). In the absence of pseudo-epitheliomatous hyperplasia a relatively uniform acanthosis, usually associated with hyperkeratosis and focal parakeratosis, was present. Occasionally the hyperkeratosis took the form of prominent keratinous plugs of follicles. The acanthosis was of a nonspecific variety but in some lesions simulated that found in neurodermatitis.

### *Dermal Infiltrate*

The dermal inflammatory reaction was in most instances characterized by concentrated masses of cells involving the entire thickness of the dermis and part of the hypodermis, or distributed in dense patches in various portions of the dermis. The reaction showed no predilection for the cutaneous appendages but was often primarily collected about blood vessels. Whereas pseudo-epitheliomatous hyperplasia was accompanied by abundant dermal reaction, the reverse was not necessarily true. The important features of the infiltrate included: (1) Its density and usually great extent. (2) The presence of numerous mature eosinophilic leukocytes reaching a concentration of as many as ninety per high-power field. (3) The large numbers of plasma cells which varied greatly in size, many with two nuclei and rarely three, and some of them indistinguishable from the giant plasma cells of mycosis fungoides. (4) The abundance of histiocytes with cytoplasm which appeared to have undergone diffuse hydropic swelling and reacted negatively to stains for glycogen and fat. Mast cells were not increased. (5) Finally, there were noted conspicuous lymph follicles

(5 of the 20 cases) with definite germinal centers. It is of interest that eosinophilic leukocytes were excluded from the germinal centers despite their presence in large numbers in the adjacent infiltrate.

In addition, mitotic figures were observed in histiocytes in 5 of the lesions; karyorrhexis with phagocytosis of chromatin was common. Binucleate histiocytes with partially overlapping nuclei closely resembling the Sternberg-Reed cells of Hodgkin's disease also were found occasionally. In 3 lesions subepidermal edema, almost urticarial, was present; in only one of these was the process of short duration (Fig. 14). In 3 instances there were subendothelial edema, swelling of endothelial cells, and a few polymorphonuclear leukocytes in the intima of dermal arteries and veins, a reaction reminiscent of one form of allergic inflammation of vessels. In 4 other lesions (1 tick, 1 mosquito, and 2 unidentified) epidermal inclusion cysts were found in the mid-dermis in the core of the infiltrate. In one lesion portions of the tick actually were included in the center of an epidermal cyst (Figs. 11 and 12). In 2 cases there was noteworthy proliferation of the squamous cells lining these cysts. Foreign body giant cells were present in the vicinity of the cyst, possibly representing a reaction to the keratin of the epidermal inclusion or to broken off remnants of the arthropod. The apparent integrity of the cyst with no obvious extrusion of keratin and the association of eosinophilic leukocytes with the giant cells suggest that portions of the arthropod or its products might be the inciting agent.

### *Comparative Lesions*

The acute lesions of the eschars of scrub typhus of approximately 9 to 27 days' duration differed strikingly from the reaction to other arthropods, essentially in the almost complete absence of eosinophilic leukocytes from the dermal infiltrate.[5] In addition the constant superficial necrosis of the eschars was not noted in the lesions under current study. On the other hand the dermal reaction to the larva of Ascaris and to the ovum of *Schistosoma japonicum* was in basic respects similar to the bites of arthropods although the pseudo-epitheliomatous hyperplasia was lacking (Figs. 23 and 24).

## Discussion

### *Differentiation from Lymphoblastomas*

The basic histologic picture of the cutaneous reaction to a variety of arthropods is a dense dermal infiltration consisting principally of mature eosinophilic leukocytes and plasma cells admixed with histiocytes which are occasionally in mitosis or binucleated, resembling Sternberg-Reed cells. This histologic reaction has been confused with

Hodgkin's disease and mycosis fungoides, a possibility noted previously by others.[6] The occasional addition of circumscribed collections of lymphocytes, sometimes with large lymphoid follicles and actual germinal centers, may suggest giant follicular lymphoblastoma, Spiegler-Fendt sarcoid, or an ill defined atypical lymphoblastoma. The presence in histiocytes of mitotic figures, however rare, serves further to camouflage the true diagnosis inasmuch as mitotic figures in dermal infiltrates must be regarded as presumptive evidence of neoplasia until proved otherwise. The reaction to the bite of arthropods is a definite exception to this rule. Furthermore, contrary to the observations of others,[6] these studies indicate that the reactions to the bites of arthropods may persist as active dermal "eosinophilic granulomas" for at least 2 years, and perhaps longer. It is precisely this fact, hitherto obscured, which requires that these lesions be considered in an altogether new stratum of differential diagnosis characterized by diseases of relatively long duration, particularly neoplastic processes. It bears noting that while the existence of a single lesion may to some degree support the diagnosis of insect bite as against neoplasm, this type of evidence must be tempered by the fact that lymphoblastomatous involvement of skin may appear as an isolated lesion for a long time. Conversely, insect bites may be multiple.

Hodgkin's disease may cause the most difficulty in differential diagnosis. The main basis for the histologic differentiation of the insect bites from the neoplastic lesions and Spiegler-Fendt sarcoid (whatever its nature) is the abundance of mature eosinophilic leukocytes in association with plasma cells. Evidence of phagocytosis is additional presumptive, but not conclusive, evidence against the diagnosis of lymphoblastoma. However, there are other criteria which lend further aid in differential diagnosis. Frequently the reaction to the arthropod is accompanied by a degree of pseudo-epitheliomatous hyperplasia of the epidermis that may be mistaken for squamous cell carcinoma. Such a reaction in conjunction with the suspected dermal infiltrate is, *per se*, potent evidence in favor of the reaction to an arthropod. Moreover, the presence of epidermal inclusion cysts, especially with foreign body giant cells intermingled with eosinophilic leukocytes, even in the absence of identifiable parts of the arthropod, is significant evidence of the inflammatory basis for the lesion.

### *Differentiation from Other Eosinophilic "Granulomas"*

Perhaps the source of greatest confusion lies in the recently publicized group of "eosinophilic granulomas of the skin."[3,4,7] There can be little question of the heterogeneity of this group in which there

appear to have been lumped lesions due to parasites of various sorts as well as to biting insects and acarines, lipoidal and reticulohistiocytoses including Letterer-Siwe's disease, periarteritis nodosa, and lymphoblastomas, particularly Hodgkin's disease. Surely, therefore, the tendency to consider the cutaneous "eosinophilic granuloma" an entity will serve only to add confusion. The differential histologic characteristics mentioned above, namely, eosinophilic plasmacellular infiltrate, epidermal inclusions, and pseudo-epitheliomatous hyperplasia, may aid the very practical purpose of segregating the reactions to the bites of arthropods from the other types of so-called eosinophilic granulomas. In the absence of pseudo-epitheliomatous hyperplasia, the reaction to the intracutaneous larvae of worms, often in the clinical form of "creeping eruption," may be indistinguishable from that of insect bites. The dermal eosinophilia in some cases of periarteritis nodosa may be fully as marked as in the lesions due to arthropods but the vascular change in the latter is relatively mild and does not present the fibrinoid degeneration that characterizes periarteritis nodosa, or, for that matter, erythema elevatum diutinum, another disease that has been included in the differential gamut. Various stains for fat that are clearly positive serve to rule out insect bites in favor of the lipoidal histiocytoses. However, the vacuolated cells of the former may be mistaken for lipoidal histiocytes in routine sections, although the granularity and fine vacuolization of the histiocytes is more evident in the histiocytoses, both lipoidal and nonlipoidal. The frequent presence of eosinophilic leukocytes in the histiocytoses calls for nice judgment in deciding that the histiocytes constitute the primary cellular response and, generally, are structurally different from those found in reactions to arthropods. Because of the identity of the names, the eosinophilic granuloma of skin has naturally been compared with the corresponding lesion of bone.[7] Here again, in the lesion of the bones, the histiocytes with the acidophilic, finely granular, often lipoid-filled cytoplasm appear to be the essential, primary cellular matrix rather than the eosinophilic leukocytes, however abundant they may be. This is not meant categorically to deny any possible relationship between eosinophilic granuloma of bone and other forms of cutaneous eosinophilic granulomas of skin, exclusive, of course, of those due to arthropods. In the previously reported mixed group of "eosinophilic granuloma,"[8] an eosinophilia of the peripheral blood commonly was present and was often at a high level. Data on the level of eosinophilic leukocytes in patients with arthropod bites included in the current study are incomplete, but in the 2 cases in which counts were made, no peripheral eosinophilia was present.

### *Pathogenesis*

No constant or significant differences were noted in lesions varying in duration from 3 weeks to 2 years. Possibly a study of a larger series of cases in the early stages may modify this impression. In only one instance (mosquito bite) was a scar formed. The duration of this lesion was unknown. In any case, it is indisputably true that the prominence of eosinophilic leukocytes and plasma cells was as marked or even greater after 1 to 2 years as after 3 weeks. This constancy in the quality of the reactions notwithstanding their duration, which is at sharp variance with the observations of others,[2,6] is of considerable interest because it implies that the stimulating agent of the arthropod or its venom in some way manages to maintain its activity over many months. This activity is achieved in the absence of any recognizable remnants of the arthropods, although in several instances there was a history of incomplete removal of a tick. Moreover, the duration of vesiculation observed in more than one-third of the cases appeared to be in days or, at most, weeks rather than in months. The pathogenesis of this acute or subacute vesiculation, occasionally recurrent in a chronic lesion, remains to be explained on a basis more adequate than mere excoriation. Inasmuch as these vesicles are of eczematous types, action of the persistent allergen within the lesions seems a likely basis for their development. There was no correlation between the occurrence of vesiculation and the duration of the lesions.

In contrast to the reactions of the skin to ticks, mosquitoes, and chiggers is the histologic picture of the eschar or primary lesion of scrub typhus (tsutsugamushi disease). In the eschar, eosinophilic leukocytes are absent or rare. This disparity is of interest because of the close taxonomic relationship of the larval mite of scrub typhus (*Trombicula akamushi* and other species) to the chigger (larval mite of *Trombicula irritans*). Of course, in the eschar there is the added factor that rickettsiae are included in the lesion, but these organisms are said not to affect the histologic reaction qualitatively.[8]

One of the lesions, of 15 months' duration produced by a tick, occurred in a Negro. Histologically, there was no appreciable epidermal reaction but considerable dermal infiltration. The reaction did not differ basically from those in the white patients, which is of interest in view of the known resistance of the skin of the Negro to reactions to lice, mites, and other arthropods.

### *Pseudo-epitheliomatous Hyperplasia*

Finally, there remains the interpretation of the principal epidermal reaction or pseudo-epitheliomatous hyperplasia. The term pseudo-

epitheliomatous hyperplasia is used commonly in the dermatologic literature, but appears aptly chosen notwithstanding the resistance to it by general pathologists. The term does not connote a "precancerous" state. It refers simply to hyperplastic epidermis which sends shoots of branching rete pegs deep into the dermis so as to *simulate* a squamous cell carcinoma. This simulation becomes especially marked when islets of epidermis appear invasive; actually they belong to the tentacular epidermis which has been cut obliquely. This reaction, which occurs often at the margin of chronic cutaneous ulcers, was observed frequently in the reactions to arthropods and usually was mistaken for carcinoma. It is stated in the literature [9,10] that the bites of insects have produced carcinomas. There is also a report of melanoma following a tick bite,[11] but this case appears to have had incomplete histologic study. However, the demonstration of a causal relationship in the bite of the arthropod to the development of the neoplasms, while of considerable fundamental interest and by no means disproved, still requires more direct evidence than is afforded by the available data.

## Summary

A histologic study was made of the reactions to the "bites" of ticks, chiggers, mosquitoes, and unidentified arthropods.

The reaction, which consisted of a dense dermal infiltrate characterized by large numbers of eosinophilic leukocytes, plasma cells, and histiocytes, may be mistaken for Hodgkin's disease, mycosis fungoides, atypical lymphoblastoma, histiocytoses, and the heterogeneous group of "eosinophilic granulomas."

The lesion is often associated with a pseudo-epitheliomatous hyperplasia which may be confused with squamous cell carcinoma. The association with an eosinophilic dermal infiltrate and with epidermal inclusion cysts provides helpful differential clues.

With one exception, no basic difference was noted in the histologic reaction of the skin to the various arthropods studied. The striking exception is the almost complete absence of eosinophilic leukocytes in the eschar or primary lesion of scrub typhus caused by the larval mite (*Trombicula akamushi* and related species).

It is emphasized that the reaction to the "bites" of arthropods may persist for many months and that, in general, no appreciable difference is noted in the histologic reaction in lesions lasting from 3 weeks to 2 years. It is therefore concluded that the stimulating agent of the arthropod somehow must persist actively in the focus of these lesions for a remarkably long time.

A single cutaneous lesion with the histologic picture suggestive of

Hodgkin's disease or other lymphoblastoma should always be suspected as having been caused by the bite of an arthropod until conclusively proved otherwise. The history of an insect bite may not be volunteered after a lapse of many months.

The cutaneous reactions of individuals, even of the same race, to different arthropods varies not only in the acute stage but also in the persistence, degree, and quality of the histologic reaction.

It remains to be determined precisely what agent in the venom or embedded parts of the arthropod, or both, is responsible for the cutaneous reaction.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

All photomicrographs were made from sections stained with hematoxylin and eosin.

### PLATE 71

FIG. 1. Pseudo-epitheliomatous hyperplasia 1½ years after an insect bite. (Army Institute of Pathology negative no. 95762.)

FIG. 2. Pseudo-epitheliomatous hyperplasia 4 months after a tick bite. (A.I.P. neg. 95767.)

FIG. 3. Pseudo-epitheliomatous hyperplasia 8 months after a chigger bite. (A.I.P. neg. 99865.)

FIG. 4. Higher magnification of the tips of the pegs of epidermis seen in Figure 3, showing numerous mitotic figures. (A.I.P. neg. 100469.)

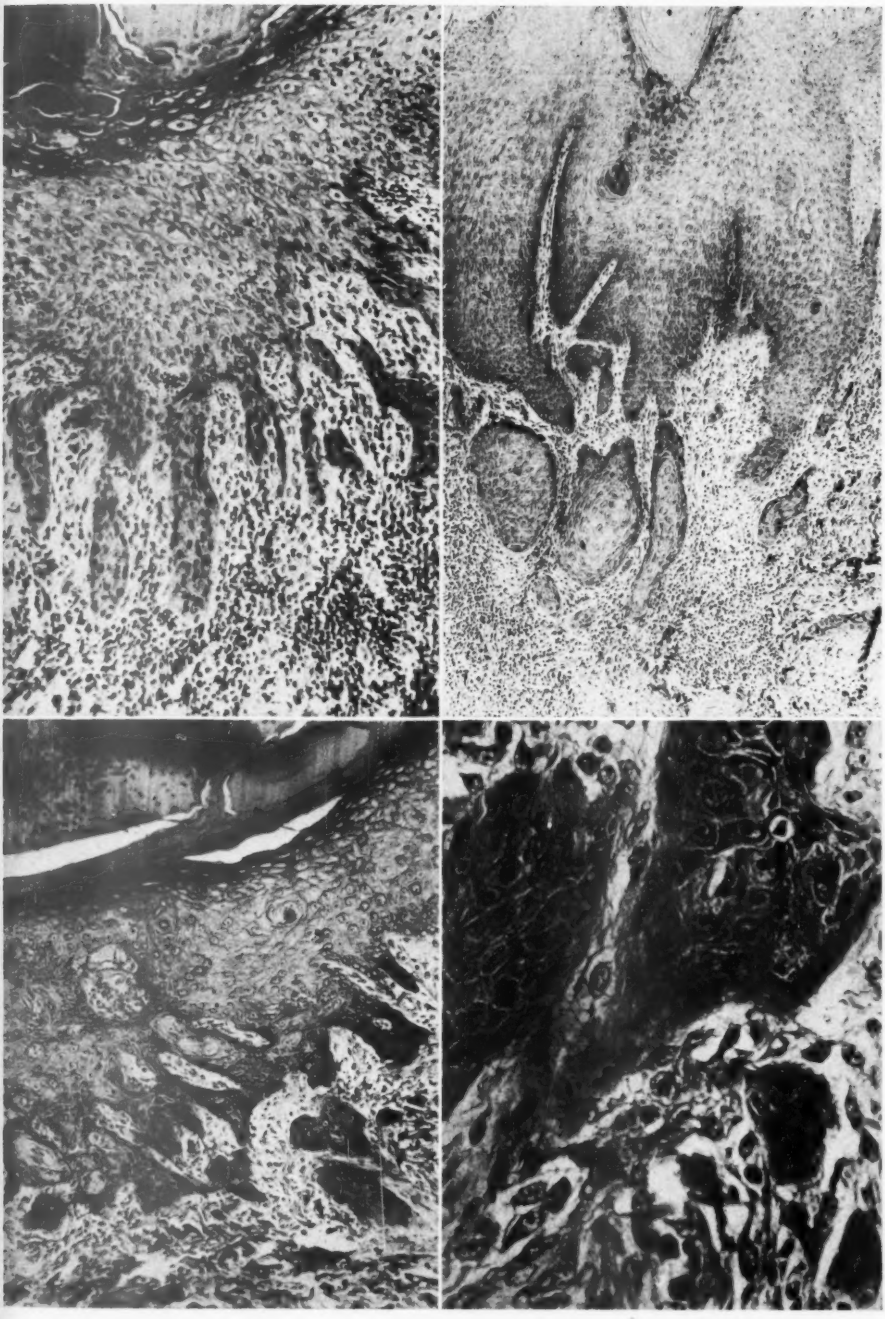

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PLATE 72

FIG. 5. Vesiculation, acanthosis, and dermal reaction 4 months after an insect bite. (A.I.P. neg. 99868.)

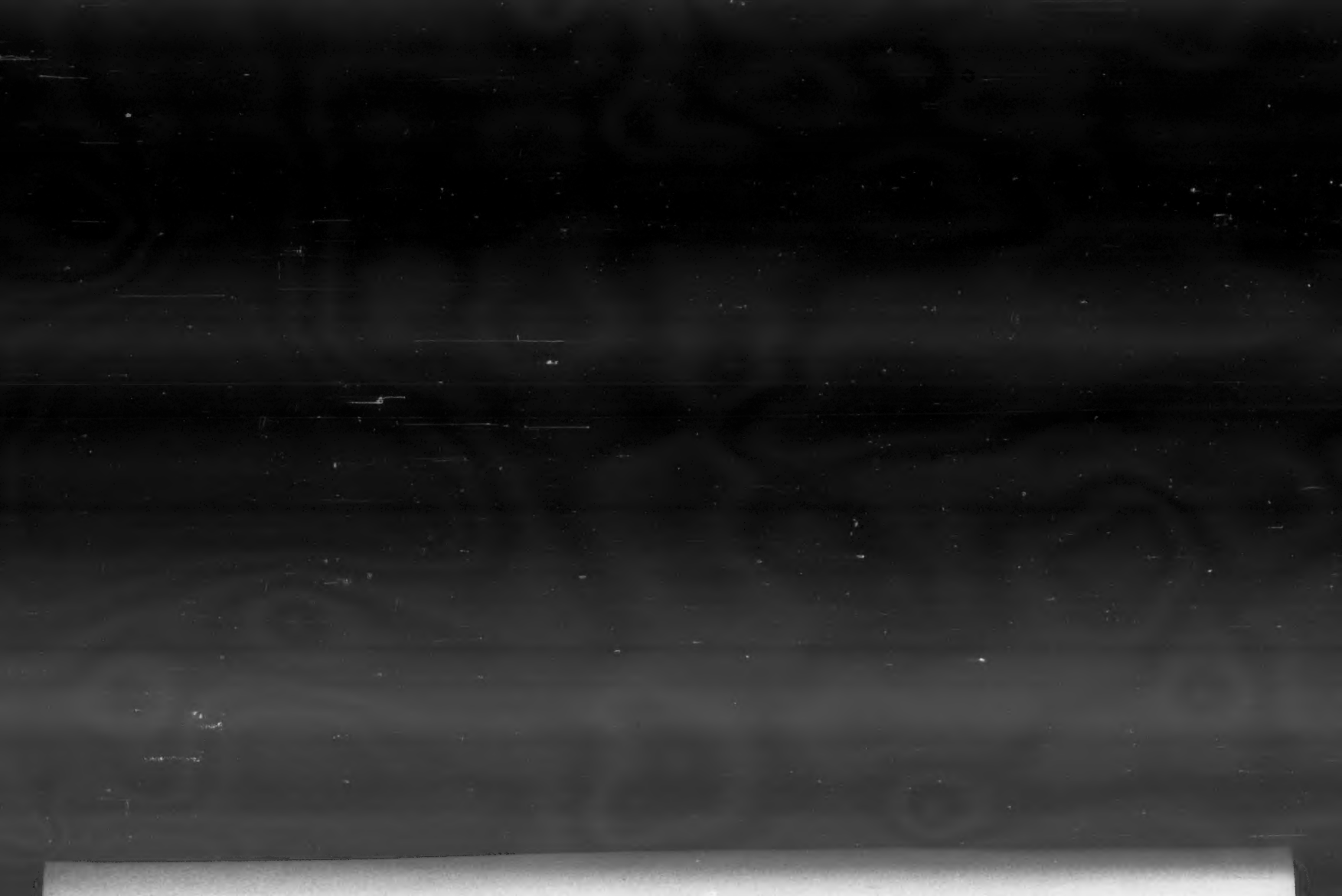
FIG. 6. Spongiosis and epidermal transmigration of eosinophilic and neutrophilic leukocytes 4 months after a tick bite. The dermal infiltrate is conspicuous. (A.I.P. neg. 100435.)

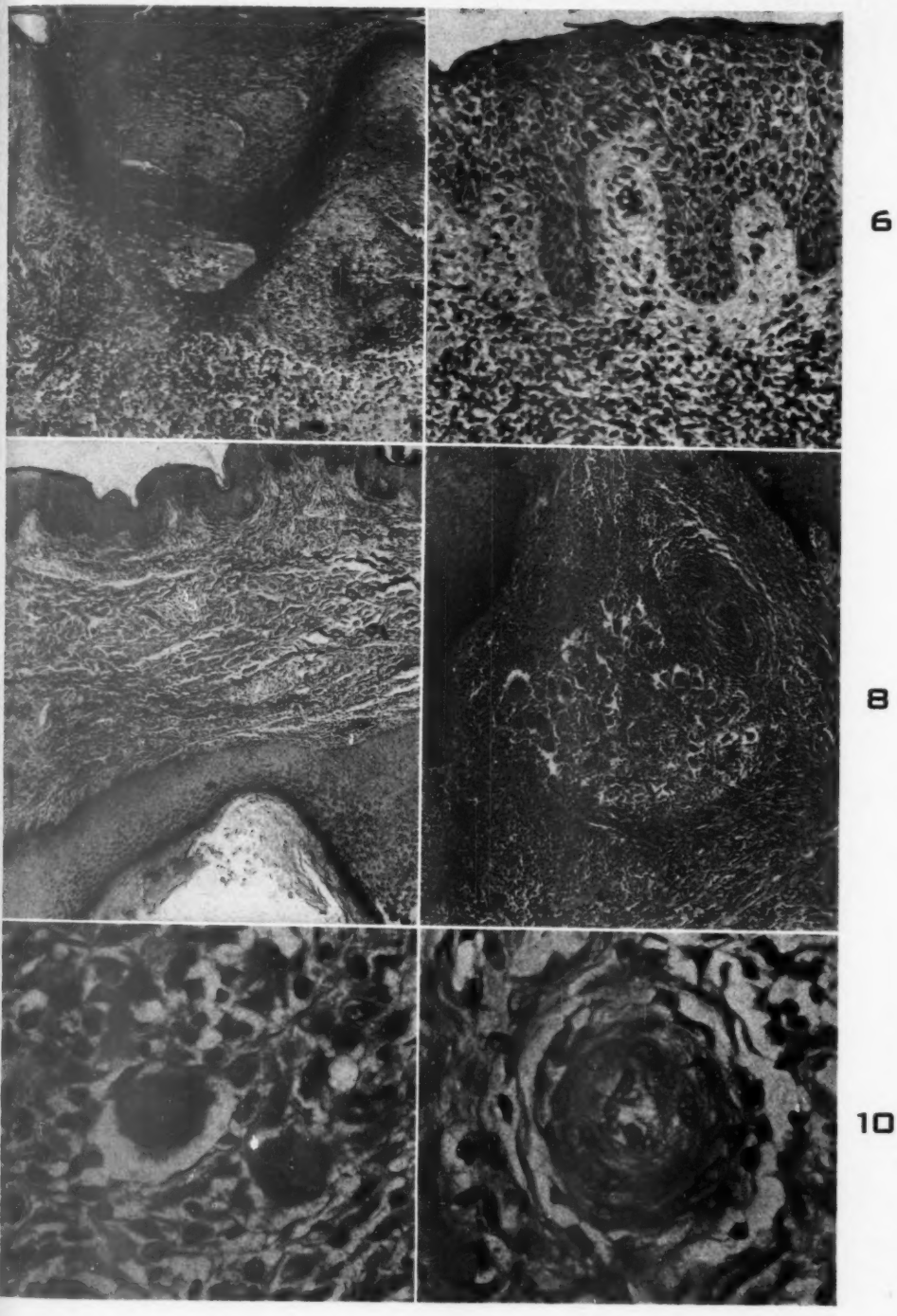
FIG. 7. Epidermal inclusion cyst 7 weeks following a mosquito bite. (A.I.P. neg. 99869.)

FIG. 8. Giant cell proliferation together with plasma cells, eosinophilic and neutrophilic leukocytes comprise the reaction to the epidermal cyst seen in Figure 7. No parts of the insect were detectable. (A.I.P. neg. 100442.)

FIG. 9. Giant cells admixed predominantly with eosinophilic leukocytes and histiocytes. This reaction was observed 7 weeks following a mosquito bite. (A.I.P. neg. 100471.)

FIG. 10. Arteriolitis with swelling of cells and fibers of the entire wall, 4 months after an insect bite. (A.I.P. neg. 100467.)





Allen

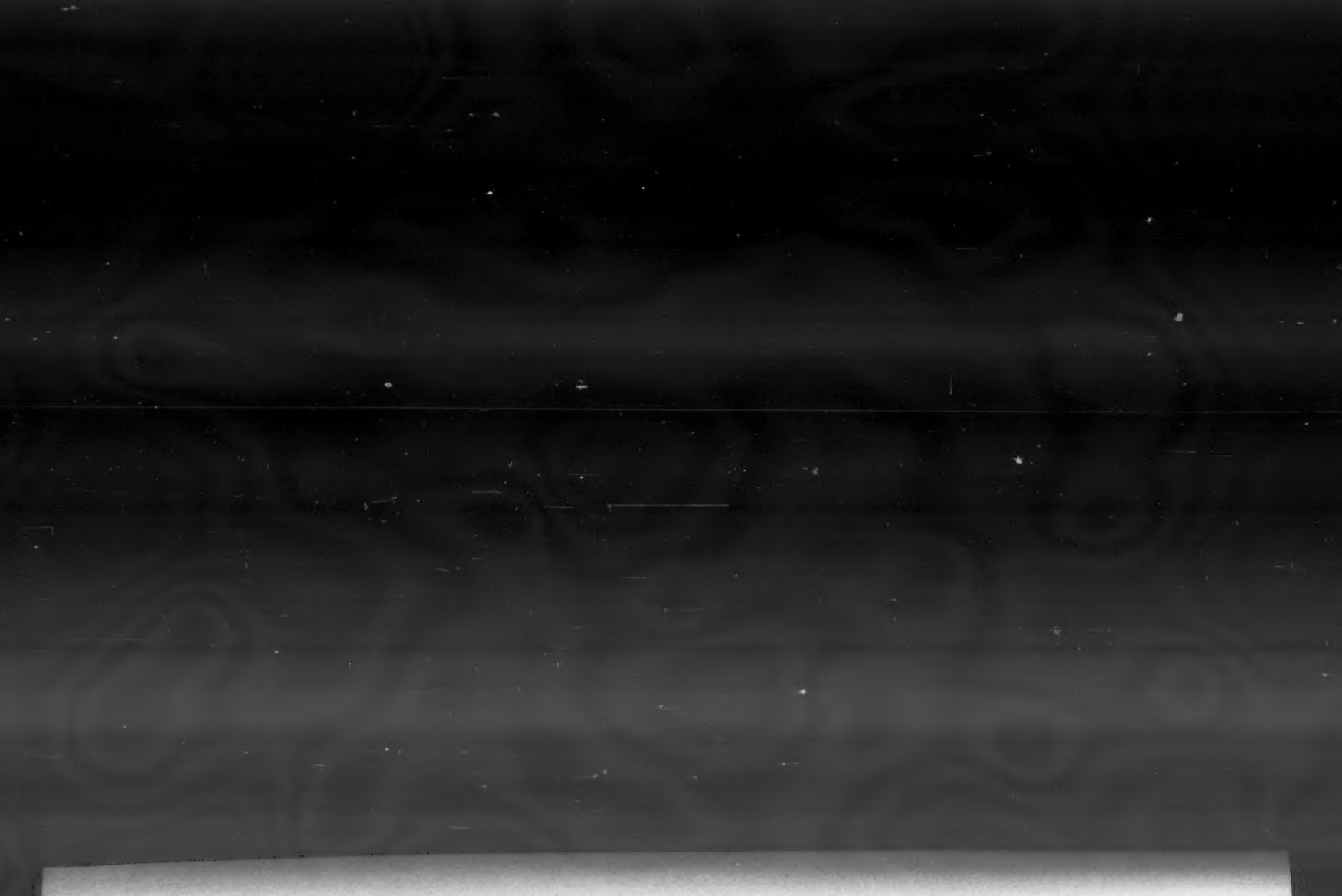
Persistent "Insect Bites"

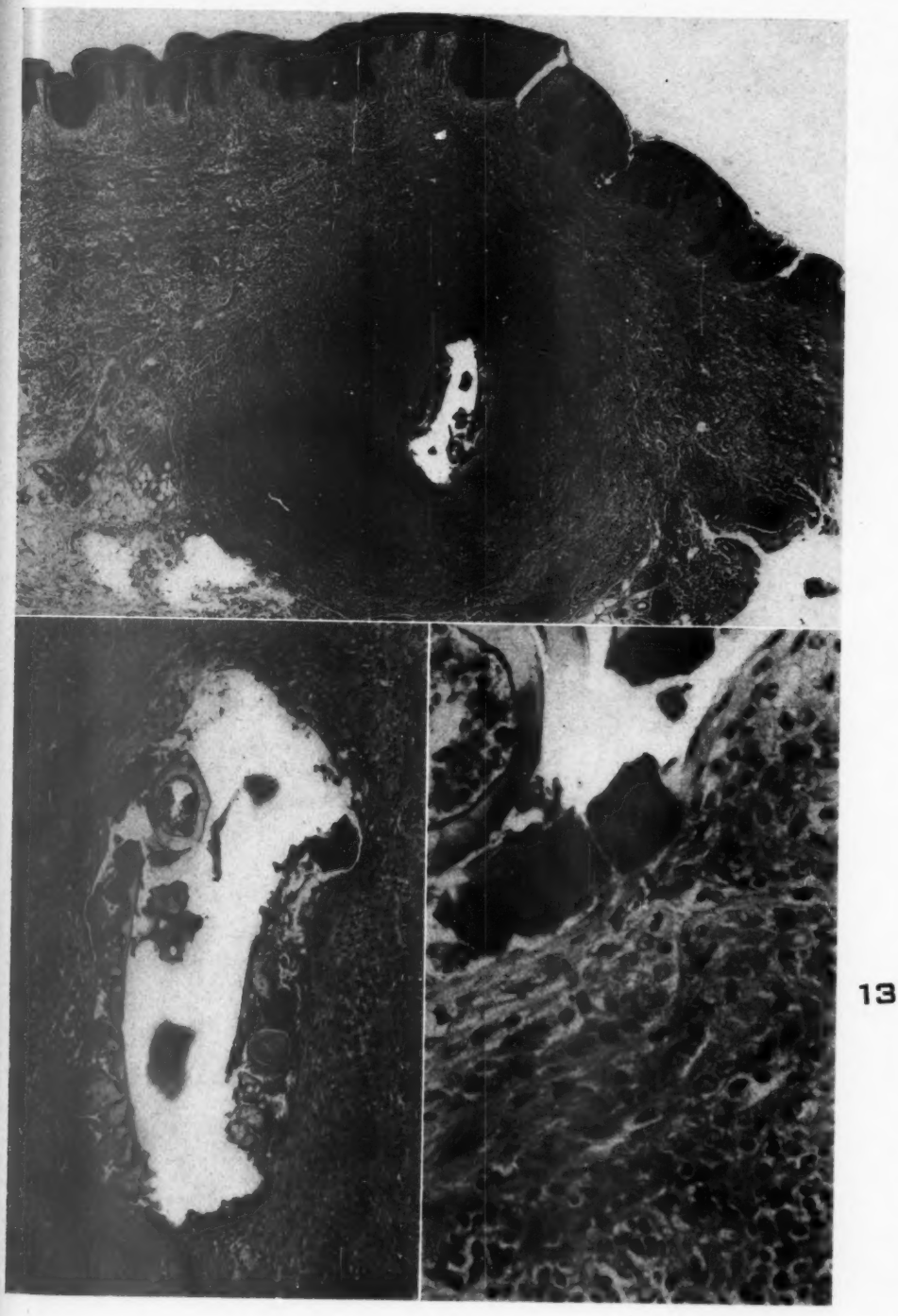
PLATE 73

FIG. 11. Reaction to a tick bite after 1½ years, showing the dense dermal infiltrate ("eosinophilic granuloma") surrounding an epidermal inclusion cyst. The contents of the cyst include portions of the tick. (A.I.P. neg. 77486.)

FIG. 12. Higher magnification of the epidermal inclusion cyst and the enclosed parts of the tick shown in Figure 11. (A.I.P. neg. 90949.)

FIG. 13. Higher magnification of Figure 12, showing parts of the tick and epithelium of the inclusion cyst, several cells of which are in mitosis. (A.I.P. neg. 100470.)





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## Plate 74

Fig. 14. Dermal reaction 4 weeks after a tick bite, showing subepidermal edema and a dermal infiltrate in which the predominant cell is an eosinophilic leukocyte. (A.I.P. neg. 100613.)

Fig. 15. Dermal reaction ("eosinophilic granuloma") to a chigger bite after 10 months. (A.I.P. neg. 95765.)

Fig. 16. Dermal polymorphous reaction ("eosinophilic granuloma") to a tick bite after 18 months. (A.I.P. neg. 78276.)

Fig. 17. Reaction to a chigger bite after 8 months. The plasmacellular response simulates a syphilitic infiltrate. (A.I.P. neg. 80169.)

Fig. 18. Large binucleated cell, and numerous plasma cells and eosinophilic leukocytes, in the inflammatory response 8 months after a chigger bite. (A.I.P. neg. 100439.)

Fig. 19. A mitotic figure and many histiocytes with hydropic cytoplasmic changes in reaction 15 months after a tick bite. (A.I.P. neg. 100466.)

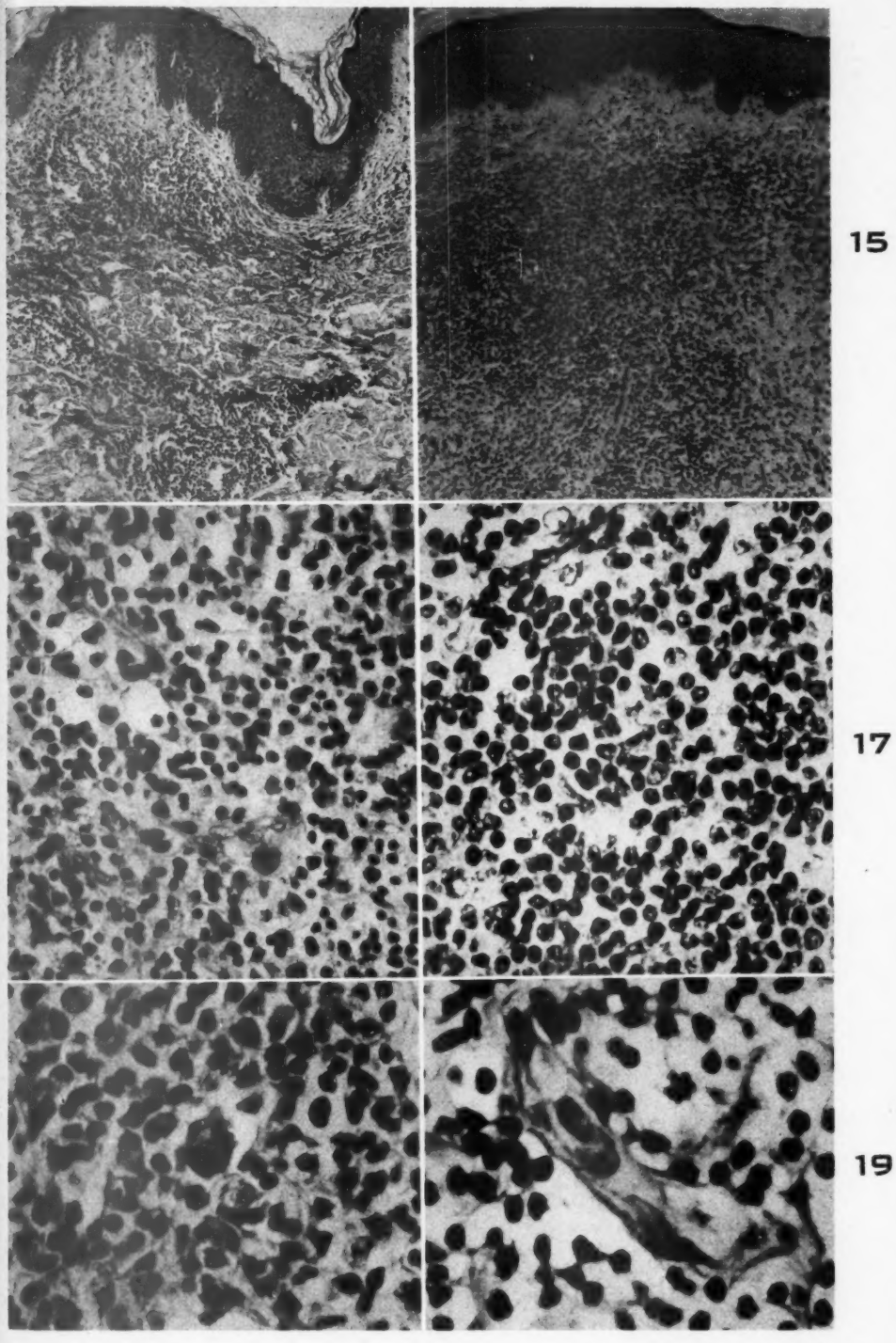

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PLATE 75

FIG. 20. Reaction 10 months after a tick bite, showing dermal infiltrate with large lymphoid follicles that have led to erroneous diagnoses, particularly of lymphoblastoma. (A.I.P. neg. 90953.)

FIG. 21. Prominent lymph follicles forming part of a dermal reaction 10 months after a tick bite. (A.I.P. neg. 100440.)

FIG. 22. From the same case as Figure 19, showing a lymph follicle in relation to sweat glands. (A.I.P. neg. 90954.)

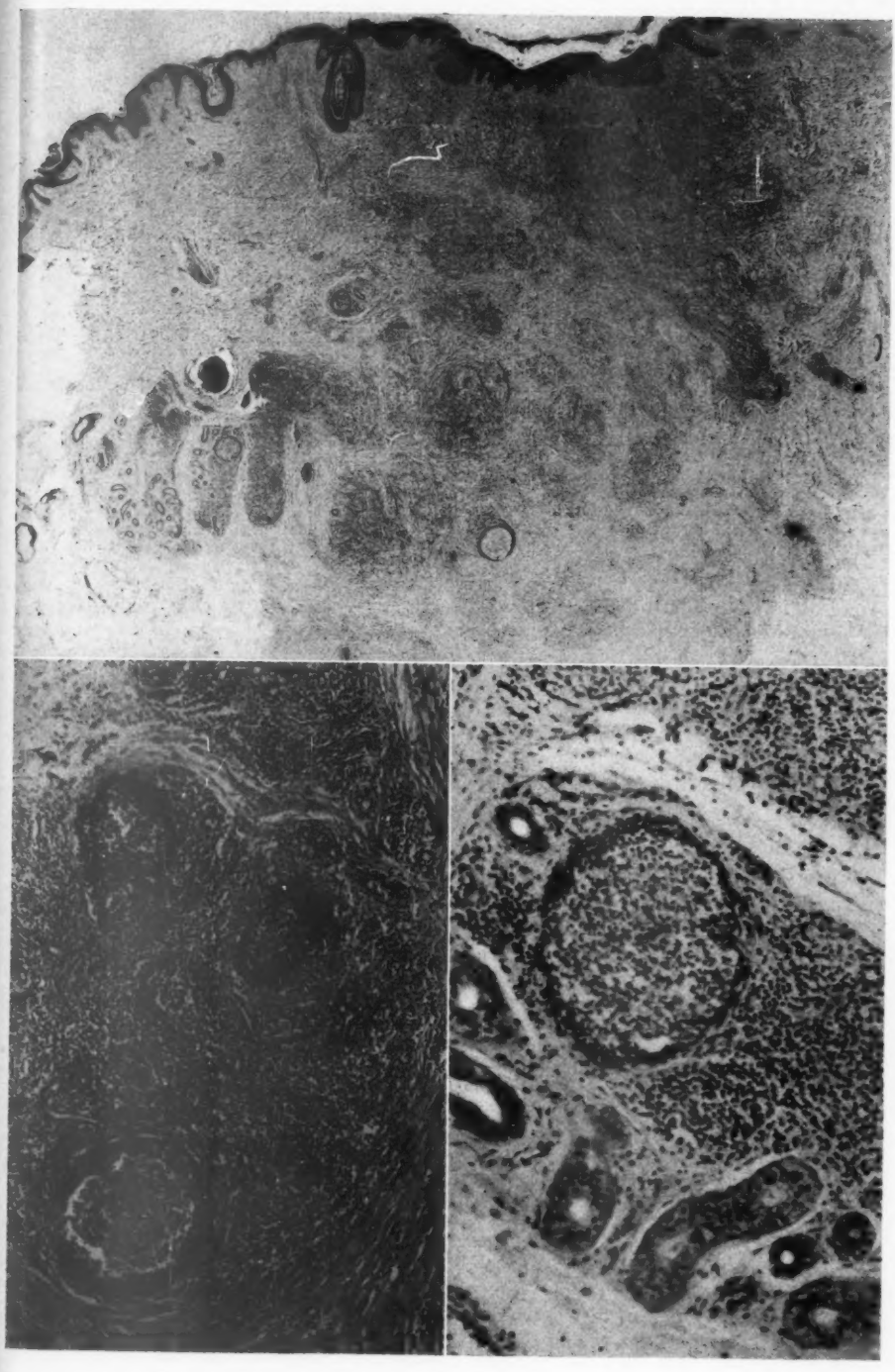

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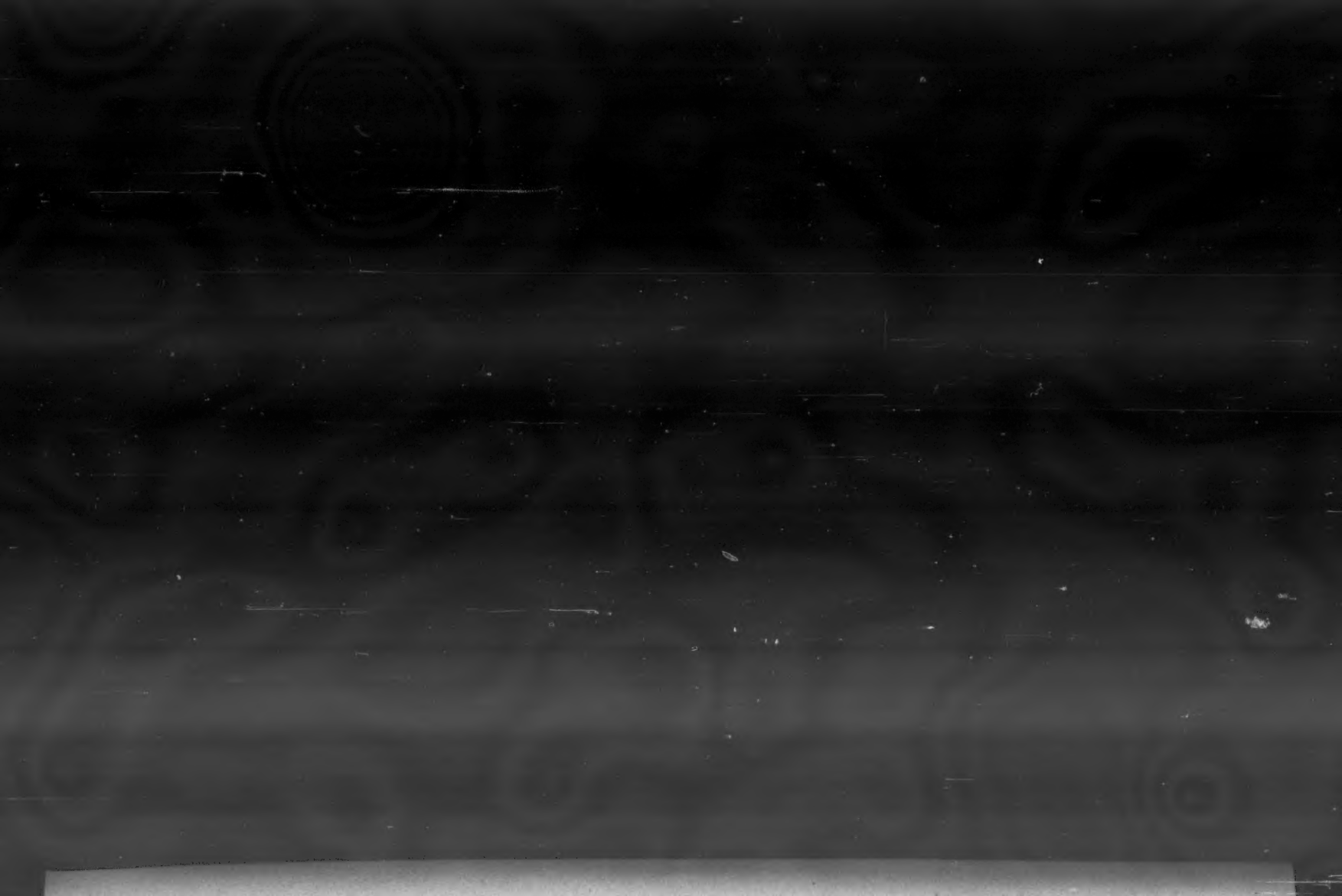
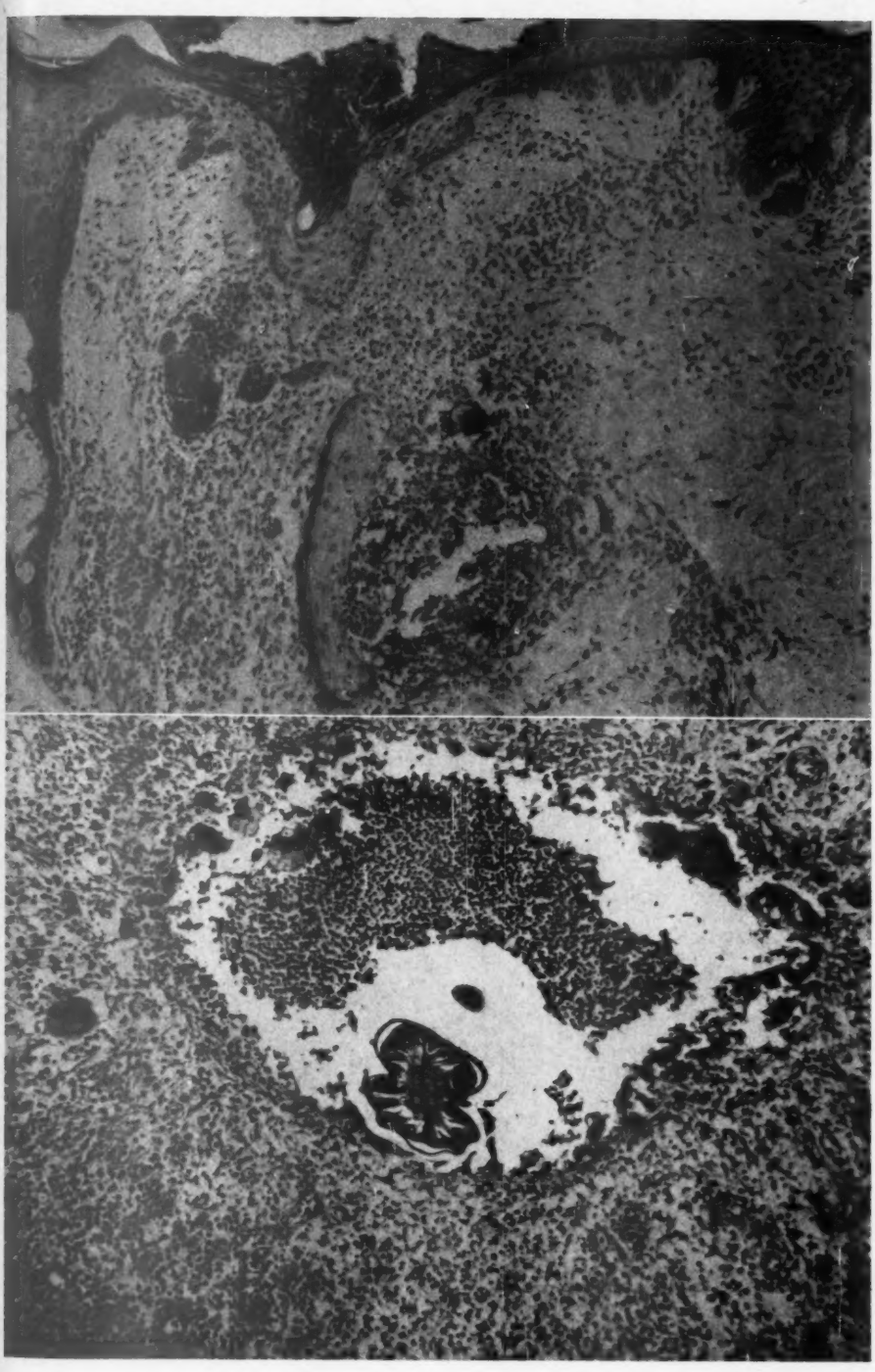
PLATE 76

FIG. 23. Dermal "eosinophilic granuloma," a reaction to the ovum of *Schistosoma japonicum*, showing superficial scaling, the ovum, adjacent epidermal inclusion, and inflammatory reaction including numerous eosinophilic leukocytes. (A.I.P. neg. 89563.)

FIG. 24. Dermal "eosinophilic granuloma," a reaction to the larva of Ascaris, showing numerous eosinophilic leukocytes and foreign body giant cells. (A.I.P. neg. 78694). (Courtesy of J. E. Ash and S. Spitz. Pathology of Tropical Diseases. W. B. Saunders Co., Philadelphia & London, 1945, 350 pp.)





Allen Persistent "Insect Bites"

# ALLERGIC HYPERGLOBULINOSIS AND HYALINOSIS (PARAMYLOIDOSIS) IN THE RETICULO-ENDOTHELIAL SYSTEM IN BOECK'S SARCOID AND OTHER CONDITIONS

## A Morphologic Immunity Reaction *

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Numerous investigations have shown a close relation between plasma cells and the pathologic globulins included in the gamma-globulin fraction and consisting chiefly of antibodies against different agents of the nature of antigens (Ranström). It may be stated here briefly that as early as 1913 Hübschmann made the supposition that plasma cells are able to produce antibodies; also, that Bing and Plum (1937) first emphasized the regular occurrence of plasma cells and other reticulo-endothelial cells in and outside the bone marrow in disorders associated with hyperglobulinemia, concluding that plasma cells are able to produce globulin themselves as has also been supposed more recently by a number of investigators.

A parallelism between hyperglobulinemia and accumulation of plasma cells in different organs, in particular in the spleen, has been demonstrated by investigations carried out by Björneboe and Gormsen (1943) on immunization of rabbits by means of polyvalent pneumococcal vaccine. These authors did not find an increase in plasma cells in hyperglobulinemia caused by injection of globulin. That hyperglobulinemia and increase of plasma cells should both be produced by the same cause, but should not otherwise be associated with one another, must be considered improbable after the demonstration by Bing, Fagraeus, and Thorell of the abundant content in the protoplasm of the plasma cell of ribose nucleotide. This substance is considered characteristic of the formation of proteins in cells, presumably giving rise to the basophilia of the cells and to their characteristic staining according to Unna's method. It has been supposed by Magnus-Levy and numerous other investigators that plasma cells in plasma cell myeloma produce the proteinic substances giving rise to hyperglobulinemia in myelomatosis.

In respect to many infections by well known bacterial agents, associated with hyperglobulinemia, it has been well established that the globulin is identical with antibody. It also has been possible during

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recent years to demonstrate hyperglobulinemia as a frequent and characteristic symptom in pathologic conditions of a more obscure nature. This applies to Boeck's sarcoid and to lupus erythematosus disseminatus. The cause of the hyperglobulinemia, its nature, and its relation to morphologic reactions have been unknown in these conditions. R. A. Moore in 1944 (page 545) stated that: "An unexplained increase of plasma globulin to 3 to 6 gm. per 100 cc. is a useful diagnostic sign" (in Boeck's sarcoid), and A. F. Coburn and D. H. Moore, in 1943 (page 213), said that "Hypergammaglobulinemia of unknown cause is a constant characteristic of disseminated lupus erythematosus."

In comparative pathologic-anatomic studies of Boeck's sarcoid, lupus erythematosus disseminatus, and other conditions, I have demonstrated, as a common feature in such disorders of different causation, a coincidence of hyperglobulinemia, paramyloidosis, or hyalinosis in the reticulo-endothelial system which is often decisive as a morphologic specific character, and is sometimes found in direct relation to an accumulation of plasma cells. In the fresh stages, precipitates of a homogeneous paramyloid substance are found, displaying transitional forms to hyaline deposits, frequently arranged in periarterial rings in the lymph nodes and the spleen. According to my observations, these changes must be considered phases of *an elementary morphologic immunity reaction with an underlying allergic hyperglobulinosis in the reticulo-endothelial system* .

The relation between these reactions in lupus erythematosus disseminatus will be described in a separate paper, while the findings in Boeck's sarcoid in particular will be discussed here. The elementary reactions, however, will be described first on the basis of a case of Letterer-Siwe's disease.

### Case 1

### *Letterer-Siwe's Disease with Hyperglobulinemia, Paramyloidosis, and Accumulation of Plasma Cells in the Reticulo-Endothelial System*

A boy, 9 years of age, was admitted to the Pediatric Ward of the University Hospital of Copenhagen (service of Dr. P. Plum) on May 6, 1946. He had had morbilli, pertussis, varicella, and rubeola. Following vaccination against smallpox at 2½ years of age, attacks of fever developed, with a rise of temperature to 41° C. of a few days' duration, the temperature then becoming normal; after a few months there was a constant increase of temperature, being normal in the morning but 38° to 39° C. in the evening. When he was 3 years old his mother observed a swelling of his right cheek and also swollen lymphatic nodes on the right side of his neck; he was found to be anemic at the same time (40 per cent hemoglobin). Since that time he had been constantly febrile, with a poor appetite and in bed for certain periods. The swelling of his cheek and of the lymphatic nodes persisted but varied much in size. In October, 1945, he had been admitted for 5 months to "Kronprinsesse Louises Barnsjukhus" in Stockholm, where a histologic examination of the enlarged lymphatic node from the angle of the jaw

gave a picture which most resembled that which is seen in eosinophilic granuloma of the bones or Letterer-Siwe's disease. Moreover, the serum albumin was found to be 1.92 per cent (the normal being 3 to 5 per cent); globulin, 6.26 per cent (the normal being 1.5 to 3 per cent); total protein, 8.18 per cent (the normal being 6.5 to 7.7 per cent); albumin/globulin ratio, 0.3 (normal, 1.5 to 2).

On examination in the Pediatric Ward of the University Hospital in Copenhagen the patient's face was found to be highly asymmetric, with considerable lateral swelling of the right cheek corresponding to the zygomatic bone. The infiltration was firm, not tender, measuring about 5 cm. in the vertical direction. Below the edge of the jaw there were lymphatic nodes. The skin showed nothing abnormal. The liver and spleen could not be palpated.

Roentgenograms of the skull showed considerable swelling of the soft parts on the right and a corresponding defect of the zygomatic arch posteriorly. There were no other alterations. The thorax, the extremities, and the pelvis displayed no abnormal signs roentgenologically. The sedimentation rate varied from 33 to 123 mm., being most frequently about 50; hemoglobin, about 60 per cent; leukocytes, 11,600 to 21,000; differential count, normal; eosinophils, 1 to 2 per cent; thrombocytes, 515,000. The Mantoux (Mendel) test was negative. No abnormal constituents were found in the urine. Total cholesterol was 175 mg. per cent; serum albumin, 3.78 mg. per cent; serum globulin, 5.11 mg. per cent; total protein, 8.89 mg. per cent.

During his stay in the hospital the patient was subfebrile for certain periods. He was discharged on June 30, 1946.

### *Histologic Examination*

Histologic examination was based on the lymph node that had been excised in Stockholm and on subsequent biopsies of a lymph node of the neck and of the tumescence on the right cheek.

In a rather well defined area in the central part of a lymph node there was an accumulation of large, pale reticulum cells with abundant cytoplasm (Fig. 1), resembling the epithelioid cells of the granulomata in Boeck's sarcoid, but without tuberculoid structure. Especially in the peripheral parts of this area were found wide, very coarse bands of a homogeneous, partially hyaline substance, surrounded by a wide border of plasma cells which also passed in dense swarms between the individual bands (Fig. 2). As already mentioned, the latter were partially hyalinized, but in other parts they were of the nature of paramyloid and did not assume a blue but a red-violet color when stained according to Mallory's method. In the more peripheral parts of the lymph node there were finer trabecular and reticular homogeneous deposits in direct relation to plasma cells and reticulum cells. The vessels also contained a precipitate of homogeneous substance in many instances, the larger and smaller vessels being surrounded by concentric homogeneous rings (Fig. 3, for comparison with the alterations in the spleen in lupus erythematosus disseminatus), between which a few plasma cells and other reticulo-endothelial cells were observed.

There were no foam cells, nor giant cells. Microscopic examination of tissue of the tumescence in the right zygomatic region showed a

uniform structure with diffuse reticulum cell proliferation with close-set cells, not especially rich in protoplasm. Reticulin staining was positive; sudan staining, negative. No necrosis, eosinophilia, foam cells, or giant cells were found.

*Histologic Diagnosis.* Letterer-Siwe's disease.

*Summary of Case 1*

Letterer-Siwe's disease with a protracted course in a 9-year-old boy began with attacks of fever following vaccination against smallpox when the boy was 2½ years old. Microscopic examination of tissue from a focus in the zygomatic region showed diffuse reticulosis, while in the enlarged lymph nodes a large central accumulation of large, pale reticulum cells, rich in protoplasm, was found. The reticulum cells resembled the epithelioid cells in the granulomata in Boeck's sarcoid, circumscribed in the peripheral parts by coarse bands of homogeneous substance, in some parts hyaline, in others of the nature of atypical amyloid and surrounded by dense swarms of plasma cells. In addition, homogeneous precipitates were found around the vessels in the form of concentric homogeneous rings resembling the periarterial alterations in the spleen in lupus erythematosus disseminatus. There was also a marked hyperglobulinemia.

On close examination of the morphologic lesions in *Boeck's sarcoid,* all of the reactions described above can be found. However, while in the case of Letterer-Siwe's disease described here they reflect only the immunity conditions in this particular case, the analogous reactions in Boeck's sarcoid form the basis of the morphologic characteristics of this disease, illustrating the different phases of their development and explaining the nature of the hyperglobulinemia.

It has been established that cases of Boeck's sarcoid generally display an increase of the serum globulin. Salvesen (1935) first drew attention to this fact. He found an increase of the total protein content in the blood in 3 cases of Boeck's sarcoid owing to an increase of the globulin fraction with an albumin/globulin ratio of 0.86 to 0.51. Harrell and Fisher (1939) and Harrell (1940) found the total proteins to be over 8 gm. per cent in all but 3 of 11 cases which they studied, and the albumin/globulin ratio was reversed in all 8 cases during the active stage. Normal values were found in one of these after recovery. Bing (1940) found an increase of the serum globulin in 2 of 4 cases of Boeck's sarcoid. Fisher and Davis (1942) presented electrophoretic patterns for the sera of 12 cases of sarcoid, all of which had been proved by biopsy. In 4 cases which showed no clinical signs of activity

the sera were almost normal, there being only a slight decrease of albumin and increase of alpha globulin. Those with active lesions were found to have a marked elevation of the gamma globulin at the expense of the albumin, frequently with moderate hyperproteinemia. This electrophoretic pattern was similar to that which has been observed in association with the formation of antibodies in response to an infectious agent.

The morphogenesis and the phasic development of the characteristic lesions occurring in Boeck's sarcoid will be considered more fully in connection with the following case, partly on the basis of findings in post-mortem material and partly on the alterations of common occurrence in material taken for biopsy.

### Case 2

### *Boeck's Sarcoid with Marked Paramyloidosis*

A woman, 28 years of age, was admitted to Medical Ward B of the University Hospital in Copenhagen (service of Dr. E. Warburg) on December 7, 1944. Her brother had died of pulmonary tuberculosis 9 years previously. The patient previously had been in good health. She was taken ill 1 year before admission with fatigue, loss of weight (15 kg.), functional dyspnea, and perspiration. She had not felt febrile and had had no pain in her side.

*Examination.* The patient was pale and lean. She had dyspnea when resting. Her tonsils were small. There was no glandular swelling in her neck, but one small gland was found in her left axilla and small glands were found in both inguinal regions. The thyroid gland was not enlarged. Upon examination of the heart, the apex impulse was felt in the fifth intercostal space inside the midclavicular line; there was no murmur; the 2nd pulmonal tone was extremely accentuated. Fine moist râles and crepitation were heard almost everywhere.

Roentgenologic examination of the lungs disclosed a large cavern in the left apex, passing through almost the entire depth of the lung. Below this there were a number of smaller caverns surrounded by fibrous adhesions. In the right lung also a large cavern was observed in the apex. On the whole, the lung was heavily infiltrated.

The temperature varied from 38° to 39° C. Hemoglobin was 95 per cent; sedimentation rate, 27; erythrocytes, 4.87 millions; leukocytes, 6,120; blood pressure, 90/40 mm. Hg. Urine: no albumin. Wassermann test of the blood, negative. No tubercle bacilli were found in repeated examinations of sputum. Direct microscopy and cultivation disclosed no tubercle bacilli. The Mantoux (Mendel) test was negative (all strengths, in repeated examinations).

On December 8 the serum protein was 7.1 per cent. Fractional determination of protein was not made.

On December 14, biopsy of the tonsil showed a tuberculoid structure (Boeck's sarcoid ?). On December 19, biopsy of an inguinal gland showed a tuberculoid structure as in Boeck's sarcoid. The patient died on January 3, 1945.

### *Post-mortem Examination*

Post-mortem examination showed the *bone marrow* of the vertebral column to be macroscopically unaltered. There were no alterations of

the *skin*. The *tonsils* were not enlarged and their cut surface was uniform. No ulcerations of the *larynx* or of the *trachea* was found.

The *pleurae* displayed extensive fibrous adhesions.

In the apex of the *right lung* a cavern was seen, measuring 3 to 4 cm. in diameter. Its walls were discolored, with irregular trabeculae and cords. The pulmonary tissue below was of nodular consistence and grayish red. The *left lung* also displayed a large cavern superiorly, measuring 6 cm. in diameter, with firm solidified areas in the walls. No miliary tubercles or peribronchitis were seen. The *bronchial glands* showed a large conglomeration of firm, only partially necrotic lymph nodes of a grayish red color, measuring 4 cm. in diameter.

The *heart* presented some hypertrophy of the right ventricle. Otherwise the myocardium was without alteration. The *lymph nodes* around the aorta formed large conglomerations of a firm consistence, having a pale grayish red cut surface. No necrotic areas were seen.

The surface of the *liver* was smooth; it was not enlarged and the cut surface was unaltered. The *suprarenal glands* showed no signs of tuberculosis. The *spleen* was enlarged, measuring 6 by 10 by 16 cm., with a dry cut surface and no tubercles. Its consistence was not so firm as in amyloidosis. The *ovaries and tubes* showed no signs of tuberculosis. The *brain* and *kidneys* were normal.

A *bacteriologic examination* was made (at the State Serum Institute) of a necrotic gland and the cavernous tissue of the right lung, of other tissue of the right lung, the spleen and a lymph node from the hilus. By this examination (comprising cultivation and inoculation into guinea-pigs) no signs of tuberculosis were demonstrated.

### *Histologic Examination*

The *spleen* was permeated, as were the *lymph nodes,* by masses of typical epithelioid cells displaying a tuberculoid structure which was well defined. In most areas giant cells were few or absent. The picture was characteristic of Boeck's sarcoid. A very extensive hyalinosis (paramyloidosis) was especially remarkable, however, being localized partly to the peripheral parts of the epithelioid-cell granulomas, in which broad, homogeneous, concentric rings, often two to four, were found (Fig. 4), and partly to certain diffuse areas in the tissue (Fig. 5). The hyaline substance extended from the periphery into the individual granulomas, which were thus in many parts wholly replaced by hyaline masses. Around these more or less transformed cell accumulations, isolated homogeneous bands and clumps were found (Fig. 6), representing a more advanced phase of hyaline or paramyloid develop-

ment from the homogeneous "extragranulomatous precipitates" outside the tuberculoid structure, which in my remaining material of Boeck's sarcoid was a characteristic finding (Fig. 8).

In their central parts the tuberculoid structures contained typical double-contoured and stratified, often calcified corpuscles, described by Schaumann, among others, as being present in tonsils and lymphatic nodes in Boeck's sarcoid. A strikingly great number of plasma cells, in addition to other reticulo-endothelial cells, were found around the epithelioid cells. There was no necrosis, but in the central parts of the granulomas a precipitate of a homogeneous substance without cellular structure was observed in several spots. In the parts of the *spleen* in which the lesions were less extensive, the follicular arteries were found to be surrounded by broad rings of a hyaline substance resembling the typical lesions in lupus erythematosus disseminatus. In the peripheral parts of these rings granulomata often were found, surrounded by hyaline bands displaying a direct continuity with the periarterial rings. The concentric rings in the transformed granulomata also bore a close resemblance to the periarterial lamellae. Staining according to Mallory's method gave very fine pictures with rings of an intense blue from which a blue network passed between the intensely red epithelioid cells and into the reticulum tissue. Just as in case 1 of Letterer-Siwe's disease, red lamellae were found scattered among the blue bands of hyaline substance, such lamellae also being conspicuous in the deep part of the splenic capsule which displayed a high degree of hyaline thickening.

Jürgens' methyl violet reaction for amyloid and Congo red staining were negative.

Tubercle bacilli could not be demonstrated.

The *lymph nodes* also displayed typical Boeck granulomata with marked hyalinosis (paramyloidosis). Here, too, concentric homogeneous rings could be demonstrated around the smaller vessels (Fig. 7), reminiscent of the periarterial lesions in the spleen in lupus erythematosus disseminatus and of the alterations in a lymph node in case 1 of Letterer-Siwe's disease with hyperglobulinemia.

The *liver* contained scattered typical granulomata with a few giant cells. No necrosis was present.

The *lungs* displayed a highly variegated picture with well defined epithelioid cell granulomata surrounded by lymphocytes and in most instances by a hyalinized tissue, in some parts passing on to large structureless masses containing epithelioid cells and giant cells, a number of which resembled Langhans' cells. Others resembled regular

giant cells of foreign body type, frequently containing the stratified, often calcified, corpuscles referred to in the description of the spleen, which were stained a deep blue by hematoxylin and eosin. Some of the granulomata resembled those of Boeck, while others could not be distinguished with certainty from tuberculosis. In the walls of the cavern an even transition from a peculiar homogeneous necrosis to hyalinosis was observed. No tubercle bacilli were found on microscopic examination. Sudan staining showed no lipoid content in the granulomata.

The kidneys, suprarenal glands, thyroid gland, intestines, appendix, and the pituitary gland displayed no alterations, especially no tuberculoid structure.

*Summary of Case 2*

Case 2 thus was noteworthy especially for the following reasons:

1. The combination of marked changes, like those seen in Boeck's disease (sarcoidosis), in the spleen, lymph nodes and lungs, with alterations in the lungs which, as far as the clinical features and the macroscopic picture are concerned, bore a close resemblance to tuberculosis (caverns), but without any tubercle bacilli being found on cultivation or microscopic examination, the tuberculin reactions also being negative. These findings may be made to correspond perfectly well with the prevailing Scandinavian view of Boeck's sarcoid as a form of tuberculosis with a high immunity (positive anergy, discussed later).

2. The combination of typical Boeck granulomata with paramyloidosis (hyalinosis), especially in the spleen, in which, in this connection, the close relation between the hyalinized border zone of the granulomata and the periarterial hyalinosis was noted in particular. This must be considered identical with the findings described above in Letterer-Siwe's disease with hyperglobulinemia, and with the lesions occurring in atypical and experimental amyloidosis, and in lupus erythematosus disseminatus.

## Discussion

The paramyloidosis in Boeck's sarcoid must be considered a definite phase of the development of the lesions, with an allergic hyperglobulinosis in the reticulo-endothelial system as the underlying primary cause. The paramyloid phase and the hyperglobulinemia in Boeck's sarcoid as well as in the other conditions mentioned must be considered an elementary immunity reaction in the reticulo-endothelial system.

In preparations from a number of other cases of Boeck's sarcoid, chiefly from lymph nodes, tonsils, and skin, I found good conformity with the view advanced here concerning the nature and phasic develop-

ment of the Boeck lesions, the morphologic structure of the lesions on the whole showing that a gradual development takes place from precipitate (Figs. 8, 9, and 10) to hyalinosis. The following features may be stressed in this connection:

1. Granulomata with large, pale epithelioid cells with *complete absence of necrosis;*

2. Outside the actual tuberculoid structure, precipitates of a homogeneous substance (*i.e., extragranulomatous precipitates*) of the same nature as that observed in the granulomata, staining in the same manner and forming homogeneous bands or clumps between the typical granulomata (Fig. 8);

3. A central homogeneous area in the granuloma, which is sometimes interpreted as slight or early necrosis, represents a similar precipitate, corresponding alterations also being observed in some instances in the walls of the vessels;

4. Preparations from cases of Boeck's sarcoid in the different phases of the disease show all transitions, from the precipitation of homogeneous eosinophilic substance (Figs. 8, 9, and 10) to marked *paramyloidosis* as in case 2, with broad concentric hyaline rings beginning peripherally in the individual granulomata (Fig. 4), which are gradually replaced by clumps of hyaline tissue (Fig. 6);

5. In most cases plasma cells in strikingly great numbers are found in direct relation to these paramyloid rings;

6. A marked periarterial hyalinosis in the *spleen* was localized to the follicular and the penicillary arteries, resembling the alterations in the spleen in lupus erythematosus disseminatus which will be dealt with in a subsequent publication.

Considering lastly the conformity with the findings in case 1 of Letterer-Siwe's disease (large, pale epithelioid cells without any necrosis; depositing of paramyloid beginning in the peripheral parts and in relation to the accumulation of plasma cells; extensive homogeneous and paramyloid deposits independent of the granulomata and giving the same staining reactions as the latter; vascular rings and hyperglobulinemia, which is a characteristic symptom in Boeck's sarcoid), I consider it highly probable that *the morphologic lesions in Boeck's sarcoid represent a serologic hyaline (paramyloid) precipitation, having as its starting point a globulin-precipitate, especially in the reticuloendothelial system.*

In a number of preparations from different cases of Boeck's sarcoid stained according to Unna's method, the epithelioid cell granulomata and homogeneous precipitates during the active stage assumed a deep

red color, in contrast with the granulomata in tuberculosis. This must be considered as a further support of the view that a hyperglobulinosis is present.

The combination of Boeck's sarcoid and paramyloidosis, so conspicuous in case 2, requires special comment. This is not, as in tuberculosis with amyloidosis, a special complication but *a phasic development of the typical morphologic lesions* characteristic of Boeck's sarcoid. It now appears that the reactions described in Boeck's sarcoid and in case 1 of Letterer-Siwe's disease, like the increase of globulin in these conditions, are quite similar to the reactions previously demonstrated in "experimental amyloidosis" after immunization, so that the view of Boeck's sarcoid advanced above as an immunity reaction in which a *hyperglobulinosis* in the reticulo-endothelial system forms the basis of the morphologic lesions (reticulosis ending in paramyloidosis) as well as of the hyperglobulinemia, can easily be made to correspond with previous findings in such immunization experiments.

Hass, Huntington, and Krumdieck, in 1943, stressed that: "It seems, therefore, that persistent or repeated stimulation of immune mechanisms is a fundamental factor in the genesis of amyloid disease." The only exception stated is the type seen in plasma cell myeloma.

Loeschcke (1927) carried out sensitization experiments in rats with a 5 per cent solution of casein sodium and, soon after the first intraperitoneal injection, found a considerable increase in the volume of the spleen, with enlarged reticulum cells which were constantly increasing in number. He presumed that antigen-antibody reacted with one another, with the formation of an insoluble precipitate. All that is termed hyalin was said by him to be the morphologic expression of such antigen-antibody fixation, whereas amyloid was perceived as a special case of the serologic precipitation of hyalin, which is present primarily only at the place of formation of the antibody, *i.e.*, in the reticulo-endothelial system. Just as the specificity of the formation of antibody against different proteins is stressed, we must, according to Loeschcke, recognize specific forms of hyalin, of which, however, only amyloidosis is open to a histologic characterization. Still, it also applies in part to the atypical amyloidosis (paramyloidosis) described here and also to the depositing of amyloid in plasma cell myeloma, termed paraproteinosis (Apitz).

As early as 1926 Letterer pointed out hyperglobulinosis, *i.e.*, the increased liberation of globulin from the cells to the tissue sap and to the blood, as the primary basis of amyloidosis (*i.e.*, experimental amyloidosis), stating that it may be caused both by the known fundamental disorders and by protein therapy, and also emphasizing the

absolute, or at least relative, increase of serum globulin in such conditions. Lastly, mention may be made of the atypical amyloidosis in serum horses described by Arndt, and others, in which "the reticulo-endothelial cell reaction" seems to be of constant occurrence. Reticulo-endothelial reactions were present at all stages, when the animal had been immunized once, while amyloidosis occurred only when the animal had been used for the production of serum for 8 months at the earliest, and was fairly constant after 16 months. According to Arndt, such reticulo-endothelial alterations may appear as a precursory stage of amyloidosis, having in the spleen a typical *perinodular* localization in the splenic follicles; the serum horse also frequently displays very marked hyperglobulinemia.

With regard to pathogenesis, structure, phasic development (reticulum cell proliferation and precipitation), localization, and alterations in the blood, the points of resemblance between such forms of experimentally produced atypical amyloidosis and the findings in Boeck's sarcoid, as described here, are so striking that we have to reckon with completely parallel processes. The morphogenetic interpretation of the alterations in Boeck's sarcoid given above tells us nothing about the etiology of the disease; it may, however, easily be made to agree with the prevailing view of tuberculosis and Boeck's sarcoid as two phases of the same disease, supported as this view is by a number of publications (Lemming, Kallós, and Warfvinge, and others).

As has been pointed out by Hellerström, a special form of anergy is present in Boeck's sarcoid in all cases, which is fundamentally different from the anergy found in the organism that is not infected by tubercle bacilli. This type of reaction (of positive anergy) is doubtless the outcome of an especially high degree of immunity; "the organism tackles the tuberculin so rapidly that no reaction, or only a faint one, appears" (J. Jadassohn).

That the morphologic reactions dealt with here must be considered allergic immunity reactions is also illustrated by the unquestionable etiologic importance of the vaccination against smallpox in the case of Letterer-Siwe's disease reported as case 1. Also, in a case of typical, histologically verified Boeck's sarcoid, described by Lemming, in which the tuberculin reaction was negative, Boeck's sarcoid developed in the skin at the site of intradermic injection of B.C.G. vaccine, Mendel's reaction remaining negative after the injection.

While the described morphologic immunity reaction (hyperglobulinosis, paramyloidosis) in Boeck's sarcoid constitutes an essential part of the morphologic characteristics of that disease, similar reactions must also be supposed to play an important rôle in a number of other

disorders. This applies to lupus erythematosus disseminatus, which will be dealt with in the article that follows, and also to syphilis, lymphogranuloma inguinale (and certain cases of tuberculosis), in which specific "necroses" and hyalinosis, often in relation to plasma cell accumulation, are of frequent occurrence, hyperglobulinemia having been demonstrated also in such cases (Bing).

In these and in other special disorders associated with hyperglobulinemia, reactions analogous to atypical (and experimental) amyloidosis must henceforward be considered decisive for the morphologic specific pattern.

## Summary

As a feature common to Boeck's sarcoid and a number of other pathologic conditions associated with hyperglobulinemia, the reticulo-endothelial system is found to contain precipitates of a homogeneous substance passing on to hyalinosis (paramyloidosis). The alterations with regard to pathogenesis, structure, and phasic development (proliferation of reticulum cells and precipitation), localization, and alterations of the blood (hyperglobulinemia) must be considered analogous to atypical and experimental amyloidosis. The common primary basis is supposed to be an allergic hyperglobulinosis in the reticulo-endothelial system, determined by persistent or repeated stimulation of immune mechanisms.

In Boeck's sarcoid the following points, among others, are thus explained:

1. The localization in the reticulo-endothelial system.

2. The morphologic features (epithelioid-cell granulomata without any tendency to necrosis; the occurrence of "extragranulomatous" precipitates; the paramyloid phase with frequently concentric, hyaline rings in the border zone; the development of a periarterial hyaline zone in the spleen and in other organs, analogous to the periarterial fibrosis of the spleen in lupus erythematosus disseminatus).

3. The occurrence of hyperglobulinemia, which is a useful diagnostic sign in Boeck's sarcoid.

4. The state of immunity, in accordance with the generally accepted view of Boeck's sarcoid as a condition with a high immunity (positive anergy).

Like the different antibodies, various forms of hyalin and paramyloid must also, after these findings, be considered products of plasma cells and other reticulo-endothelial cells.

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[*Illustrations follow*]

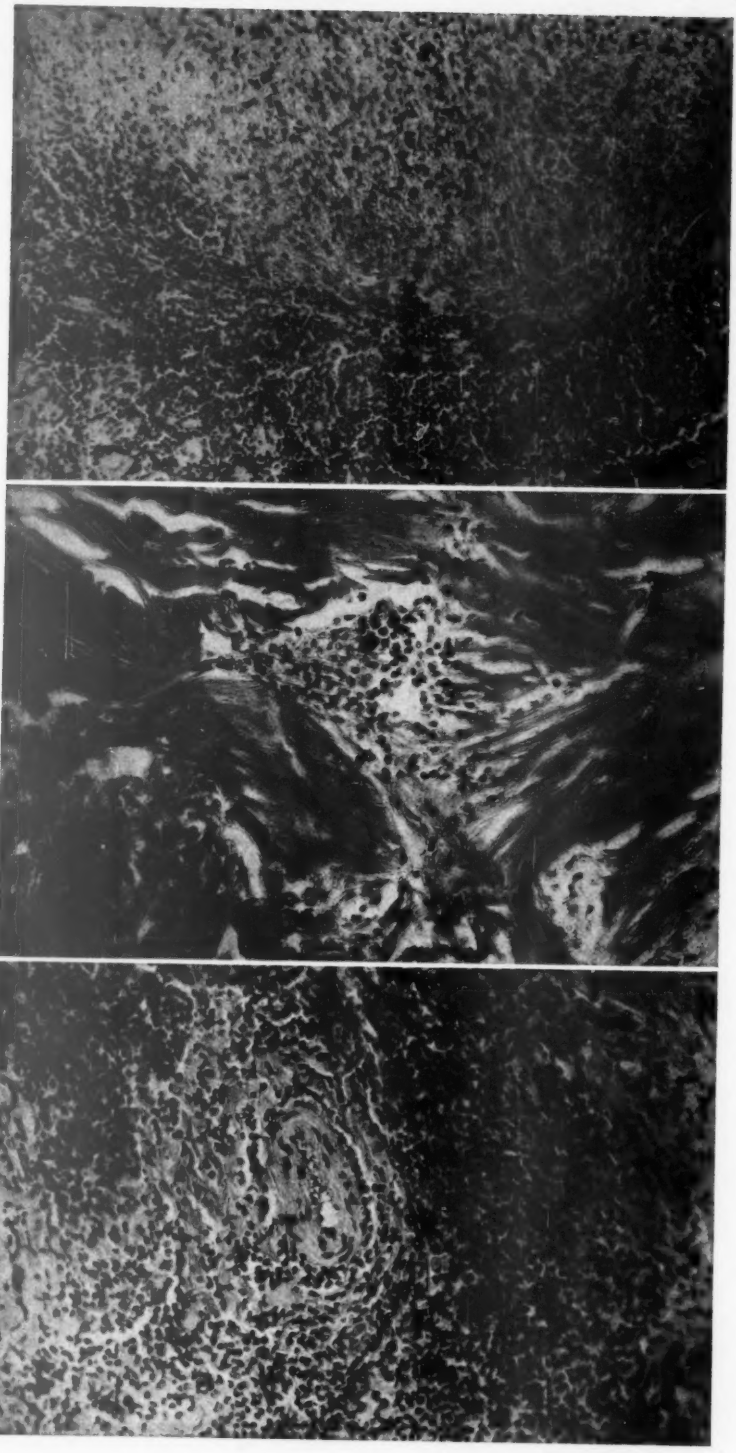
## DESCRIPTION OF PLATES

### PLATE 77

FIG. 1. Case 1. Epithelioid-cell reaction in a lymph node in Letterer-Siwe's disease with hyperglobulinemia. Hematoxylin and eosin stain. × 135.

FIG. 2. Case 1. Hyalinosis (paramyloidosis) with plasma cell accumulations in a lymph node. Hematoxylin and eosin stain. × 320.

FIG. 3. Case 1. Periarterial hyaline (paramyloid) rings in a lymph node. Hematoxylin and eosin stain. × 220.




Teilum

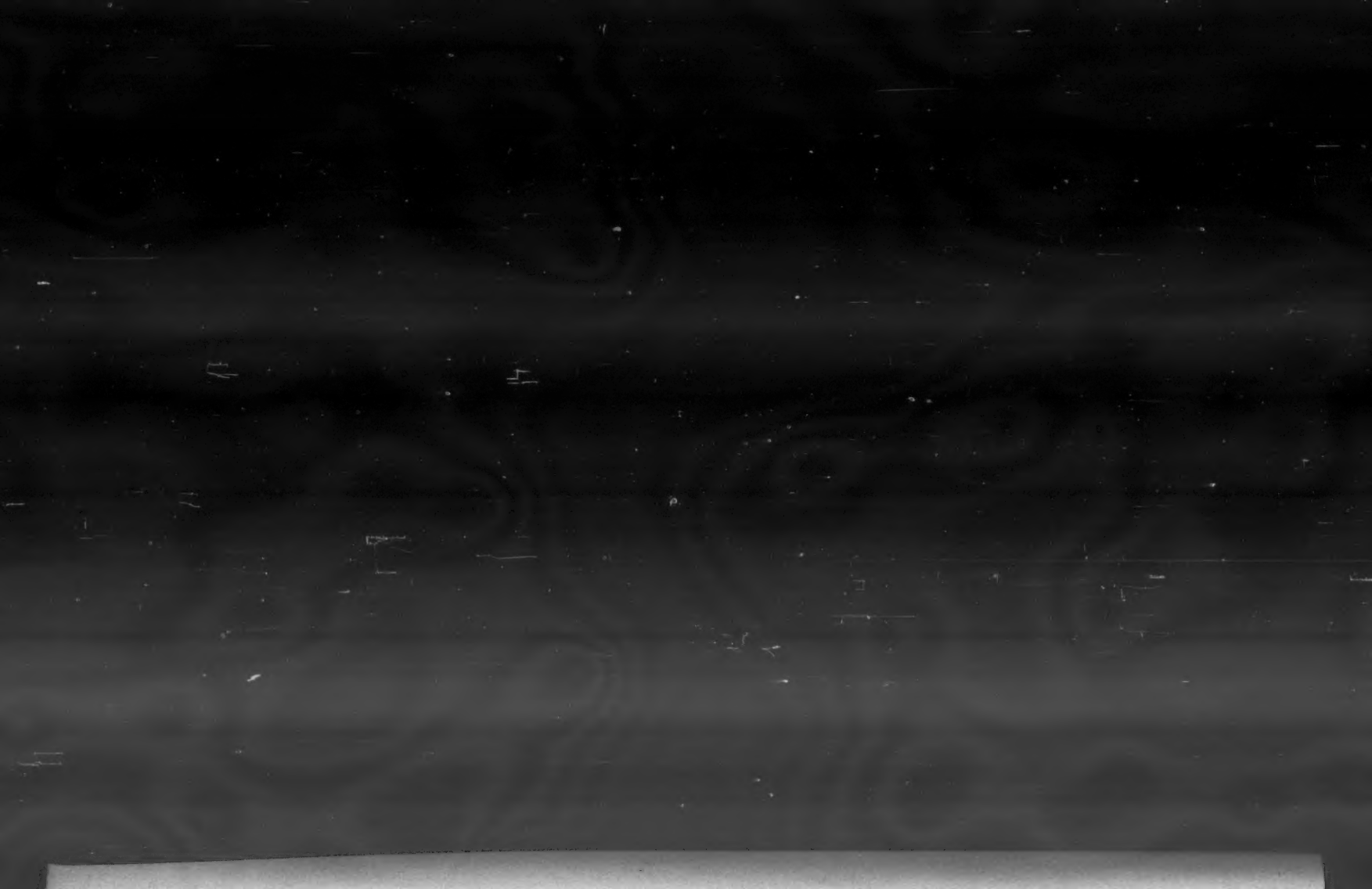
Allergic Hyperglobulinosis and Hyalinosis

PLATE 78

FIG. 4. Case 2. Lymph node. Boeck granulomata with concentric paramyloid rings in the peripheral parts. Hematoxylin and eosin stain. × 220.

FIG. 5. Case 2. Lymph node. Boeck's sarcoid, passing on to diffuse paramyloidosis. Hematoxylin and eosin stain. × 150.

FIG. 6. Case 2. Boeck's sarcoid in spleen. Paramyloidosis beginning in the peripheral part of a granuloma. In the circumference isolated paramyloid bands and clumps are present. (For comparison with the extragranulomatous precipitates in Fig. 8.) Hematoxylin and eosin stain. × 320.



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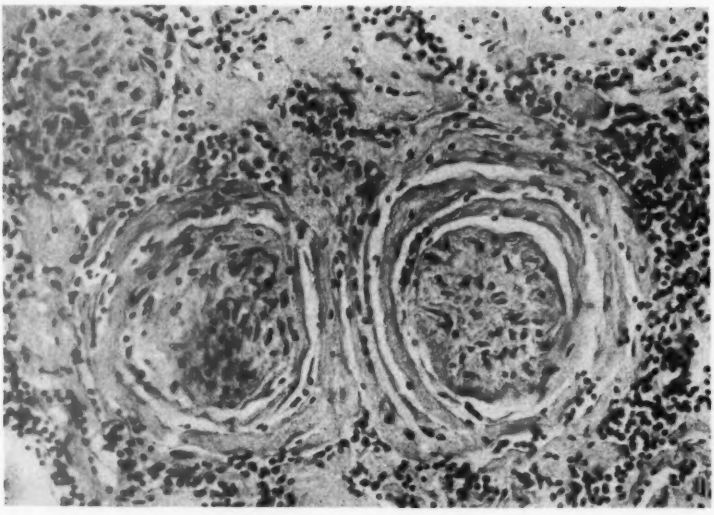

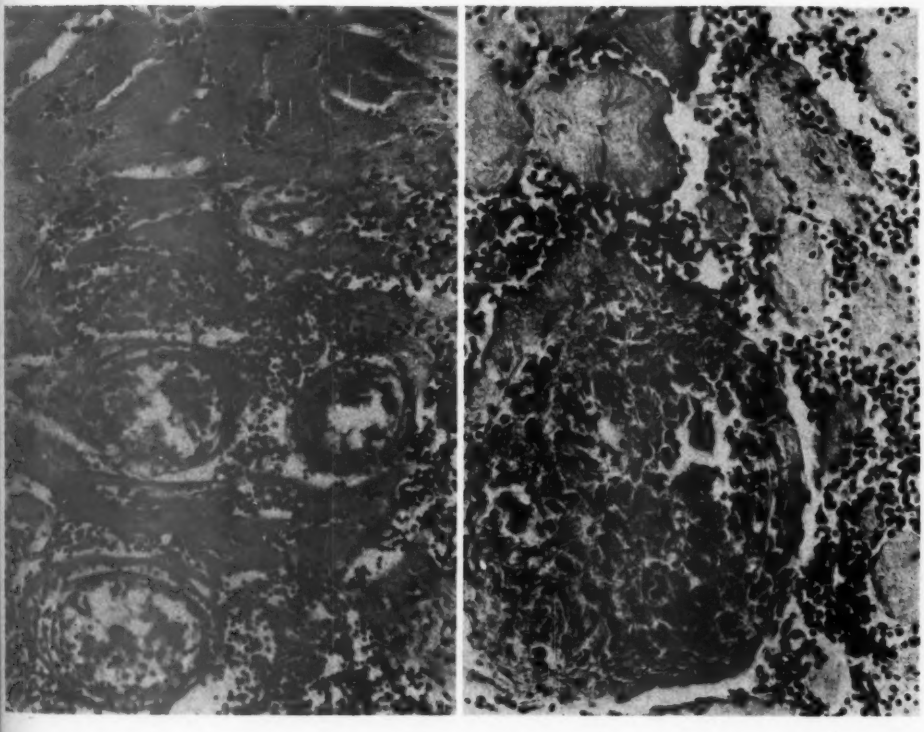

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Teilum

Allergic Hyperglobulinosis and Hyalinosis

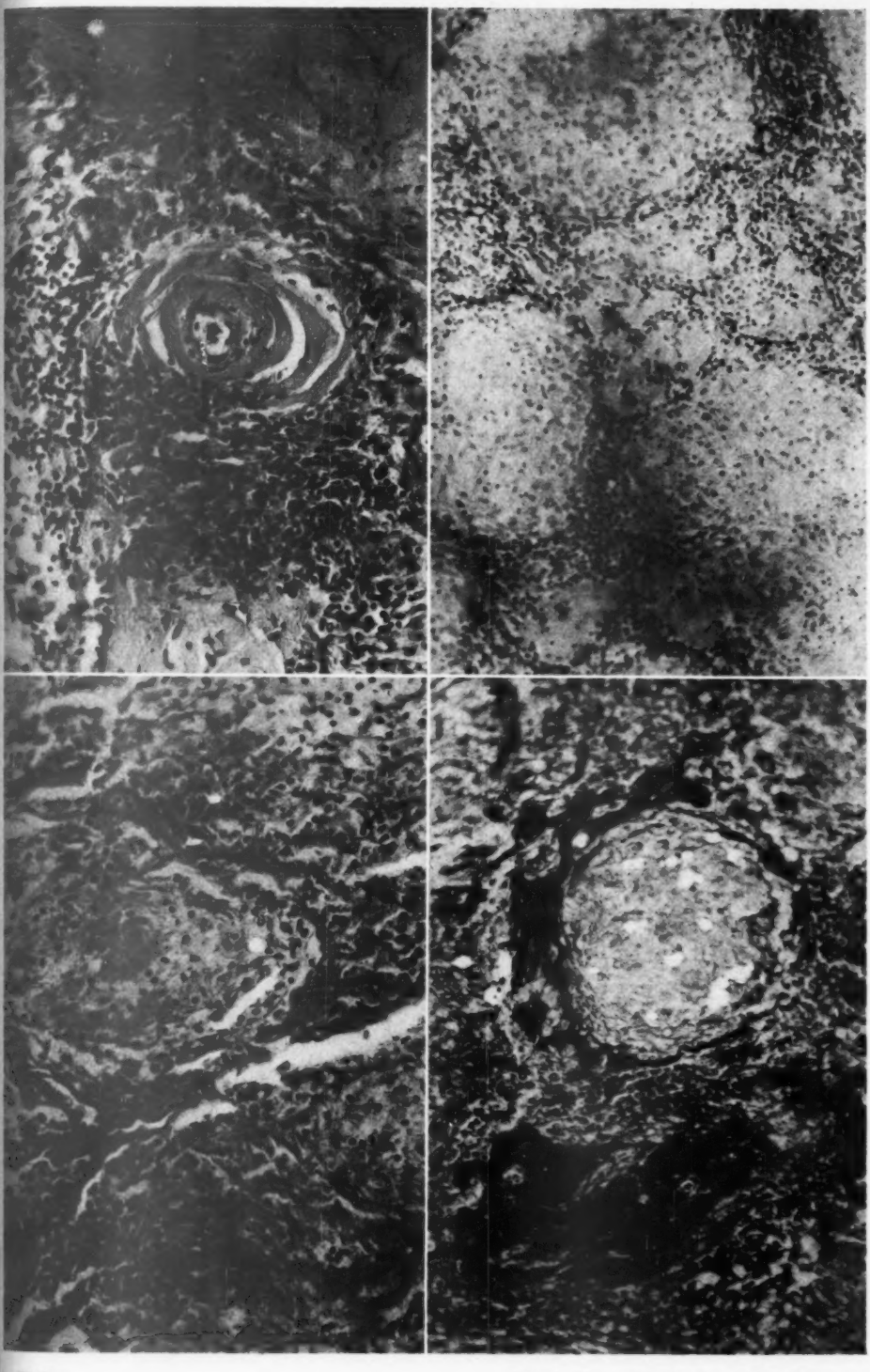
PLATE 79

FIG. 7. Case 2. Periarterial hyaline (paramyloid) rings in a lymph node in Boeck's sarcoid. (For comparison with Fig. 3.) Hematoxylin and eosin stain. × 220.

FIG. 8. Extragranulomatous homogeneous precipitates (lymph node) without hyalinosis; a common finding in Boeck's sarcoid. (For comparison with Fig. 6.) Hematoxylin and eosin stain. × 200.

FIG. 9. Extragranulomatous precipitates (lymph node, Boeck's sarcoid). Hematoxylin and eosin stain. × 240.

FIG. 10. Lymph node in Boeck's sarcoid. Homogeneous precipitates assuming a red color when stained by Mallory's method. × 240.




Teilum Allergic Hyperglobulinosis and Hyalinosis

# HYPERGLOBULINEMIA, PERIARTERIAL FIBROSIS OF THE SPLEEN, AND THE WIRE LOOP LESION IN DISSEMINATED LUPUS ERYTHEMATOSUS IN RELATION TO ALLERGIC PATHOGENESIS*

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As a common feature of Boeck's sarcoid and a number of other conditions associated with hyperglobulinemia, I have described in the preceding article (1948) the precipitation, especially in the reticuloendothelial system, of a homogeneous amyloid-like substance, passing on to hyalinosis, which, in the spleen and in lymphatic nodes *inter alia*, frequently occurs in the form of concentric rings. This is apparently an elementary morphologic immunity reaction with *an underlying allergic hyperglobulinosis in the reticulo-endothelial system.* That study emphasized the importance of this reaction in Boeck's sarcoid as an essential alteration pathogenetically related to the experimental "amyloidosis" observed after immunization. Those findings will be applied to lupus erythematosus disseminatus in the present study.

Investigations of recent years have shown that lupus erythematosus disseminatus very often is associated with hyperglobulinemia (Coburn and Moore, 1943, Thyresson, 1944) of unknown nature. Coburn and Moore (page 213) stated: "Hypergammaglobulinemia of unknown cause is a constant characteristic of disseminated lupus erythematosus."

In this disease the spleen, moreover, is the seat of a peculiar periarterial fibrosis confined to the central and penicillary arteries, which was first observed by Sacks (Libman and Sacks); it was described later by Klemperer, Pollack, and Baehr (1941) and Kaiser (1942), among others. This periarterial fibrosis, although not specific for lupus erythematosus, nevertheless is known to occur with a high frequency in this disease, as stated by Kaiser, who (page 38) further adds: "The connection between the periarterial fibrosis and the other lesions of disseminated lupus erythematosus is obscure."

It will be shown in the following study that the hyperglobulinemia and periarterial fibrosis occurring in the spleen in lupus erythematosus disseminatus are identical, pathogenetically and morphologically, with the alterations in Boeck's sarcoid and the atypical amyloidosis described in the preceding article (1948), having allergic hyperglobulinosis as a common primary foundation.

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### *Allergic Pathogenesis and Morphogenesis of Lupus Erythematosus Disseminatus*

The nature of the hyperglobulinemia and the periarterial fibrosis in the spleen in lupus erythematosus disseminatus must be considered in connection with the pathogenesis, which will therefore be mentioned here briefly. A discriminating survey of the different views of the nature of the disease, from 1872 to 1942, has been given by Libman who, in 1924, together with Sacks, described its special form of valvular and mural endocarditis. In a very thorough morphologic description by Klemperer, Pollack, and Baehr (1941), the alterations observed in lupus erythematosus disseminatus are considered "local manifestations of the widespread damage of collagen." These authors dissociated themselves from a concept of allergic pathogenesis, as they wrote: "Furthermore, the classic clinical evidences of hypersensitivity so frequently observed in periarteritis nodosa are lacking in disseminated lupus erythematosus. It would be a mistake, therefore, to speak glibly of lupus erythematosus disseminatus as an allergic disease—at least in the narrow sense of the term." The slight cellular reaction and the absence of a specific granulomatous phase are also stressed as contrasting with the ordinary findings in acute or granulomatous inflammation.

However, the miliary granulomata and nodular necrosis of focal occurrence in the serosa (parietal and visceral pleura), macroscopically visible as clustered grayish white nodules resembling miliary tubercles and described in my previous publications (1945, 1946), afford proof of the *allergic* nature of the disease; these alterations have to be interpreted as special phases of an allergic morphologic tissue reaction in conformity with the typical localization of the disease to the serous membranes. The focal allergic pneumonia described (1946), the endocarditis and the alterations in a number of other organs, such as lymph nodes, kidneys, vessels, and connective tissue, must henceforth also be regarded in the same way and may, in certain of their phases, be completely parallel with Arthus' allergic necrosis (1946). With regard to the Arthus phenomenon, reference may be made to Culbertson's demonstration (1935) of the fact that it is clearly dependent on the occurrence of circulating antibody, thus being of the same nature as anaphylaxis. After neutralization of the circulating precipitin, the phenomenon could no longer be elicited. Apart from all differences, I now see obvious pathogenetic points of resemblance with the conditions dealt with more fully in the preceding article (1948), Boeck's sarcoid and the morphogenetically related experimental atypical amyloidosis, in which "persistent or repeated stimulation of immune mechanisms is a fundamental factor in genesis."

*Hyperglobulinemia in Lupus Erythematosus Disseminatus*

Both in the conditions mentioned above and in lupus erythematosus disseminatus an increase of the serum globulin is found. In the latter disease, this is a characteristic symptom of frequent occurrence. It was first pointed out by Coburn and Moore (1943), who found an inversion of the albumin/globulin ratio in 15 patients, all of them being females between 6 and 36 years of age. In most of the cases the total protein was within normal limits. Each albumin determination was below the normal (mean value, 3.1), whereas each patient had a total globulin above the normal (mean value, 3.8) and each euglobulin value was found to be considerably increased (mean value, 1.0).

Electrophoretic analyses showed that this increase of globulin was to be found chiefly in the gamma fraction with an abnormally low albumin/globulin ratio. The other globulin fractions were approximately normal. Among other conditions in which such abnormally large quantities of gamma-globulin have been demonstrated, mention is made of the serum of hyperimmune antipneumococcal horses, Boeck's sarcoid, lymphogranuloma venereum, and certain cases of plasma cell myeloma.

*Periarterial Fibrosis of the Spleen in Lupus Erythematosus Disseminatus*

The high frequency of periarterial fibrosis of the spleen in disseminated lupus erythematosus is apparent from the literature (see Kaiser, page 31). Klemperer, Pollack, and Baehr (1941, 1942) ascertained its presence in 19 of 20 cases; Kaiser, in 15 of 18 cases.

Klemperer, Pollack, and Baehr (1941, 1942) described this lesion as a special periarterial fibrosis confined to the central and penicillary arteries, which in cross sections display concentric rings consisting of thick collagenic fibrils produced at the sacrifice of periarterial lymphatic tissue. In one of their cases many of the newly formed collagenic fibrils showed signs of fibrinoid degeneration, eosinophilic swelling and homogenization, and even basophilia.

Kaiser found macroscopic perisplenitis in 10 of 18 cases and considered periarterial fibrosis present when the periarterial collagen of the follicular and penicillary arteries, which normally is closely packed and without evidence of hyalinization, was found to be present in at least three layers, around at least half the circumference of the vessels, producing the appearance of concentric rings. Kaiser stated that this collagen was hyaline in most cases, and in others it was partially broken down to granular eosinophilic material. In most instances the majority of the smaller arteries were affected, whereas periarterial fibrosis was not found in relation to the medium or large arteries, or

around any of the venous structures. As a consequence of the fibrosis, the average diameter of the follicular vessels was found to have increased to about 125 $\mu$ in the cases affected, as compared to the normal average of 60 to 75 $\mu$.

### *Periarterial Hyalinosis in Allergic Conditions Associated with Hyperglobulinosis*

After the demonstration of similar hyaline periarterial alterations in the spleen in lupus erythematosus disseminatus and in various other disorders, I have arrived at the view that the periarterial fibrosis in lupus erythematosus disseminatus is not specific; it is essentially different from the focal allergic reactions with necrosis and granuloma formation described in the latter disease. It is a lesion of the spleen which is common to a number of different disorders which have in common the feature of displaying an *allergic hyperglobulinosis* of the reticulo-endothelial system with persistent or repeated stimulation of immune mechanisms as the fundamental factor in the genesis.

#### a. Lupus Erythematosus Disseminatus

In my previous description (1945, 1946) of the allergic lesions in the narrowest sense of the term (miliary granulomata and nodular foci of necrosis) which occur in lupus erythematosus disseminatus, the alterations of the spleen were not mentioned. Both of the cases reported (1945), however, displayed a typical periarterial hyaline zone with concentric rings. In *case 1* (no. 366/44) the follicular and penicillary arteries of the spleen were thus found to be surrounded by concentrically arranged hyaline bands (Fig. 1) forming up to five lamellae which caused the external diameter of the vessels to be more than twice normal. The individual rings were composed of large and small, often slightly wavy, disconnected hyaline lumps which assumed a red color when stained according to the van Gieson-Hansen method, and a deep blue color with Mallory's stain. When the latter staining method was employed, the vascular wall itself was seen to contain an intensely red-colored substance, and in the parts peripheral to the latter a few coarse, red, Mallory-stained filaments could be observed in relation to the blue hyaline bands, small red bands also being seen peripherally or between the hyaline rings. In the circumference and scattered between the hyaline rings numerous réticulo-endothelial cells were seen, including a few plasma cells which assumed an intensely red color when stained according to Unna's method. The hyaline substance did not give an amyloid reaction with Jürgens' methyl violet or Congo red staining.

*Case 2* of lupus erythematosus disseminatus (no. 447/44) displayed

a similar picture (Fig. 2). Here, too, scattered reticulo-endothelial cells were observed around and between the hyaline rings. With regard to the structure of the periarterial fibrosis of the spleen in lupus erythematosus disseminatus, reference may also be made to the more detailed descriptions in the articles by Klemperer, Pollack, and Baehr and by Kaiser.

### b. Periarterial Hyalinosis of the Reticulo-Endothelial System Associated with Allergic Plasma Cell Reaction

In various previous observations (Bing and Plum, Björneboe and Gormsen) a connection between an accumulation of plasma cells and other reticulo-endothelial cells and hyperglobulinemia in different pathologic conditions and immunization has been demonstrated, a fact greatly supporting the supposition that these cells are themselves able to produce globulin. The allergic hyperglobulinosis and hyalinosis (paramyloidosis) described in the preceding article (1948) as occurring in the reticulo-endothelial system and which I have considered as the basis of the alterations dealt with here must, however, be associated with this property of the cells. It now appears that whereas large accumulations of plasma cells do not seem to be of common occurrence in the reticulo-endothelial system in lupus erythematosus disseminatus, a periarterial fibrosis of the spleen and of lymph nodes, completely corresponding to the alterations in lupus erythematosus disseminatus, may be accompanied in other conditions with allergic hyperglobulinosis and hyperglobulinemia by a marked accumulation of plasma cells. In some cases an *allergic plasmacytosis* of this nature may dominate the clinical and the pathologic-anatomic pictures and may in a number of cases give rise to confusion with plasma cell myeloma or aleukemic plasma cell leukemia.

#### *Case 3*

The patient was a man, 69 years of age, who previously had been in good health, but was admitted to the hospital because of an affection of the skin resembling psoriasis. It was of 1 year's standing and, after admission, appeared as a typical lupus erythematosus. Otherwise there were no special findings in the objective examination. Sedimentation test: 104, 110, 100 mm.; Takata's test, positive; serum protein, 9 per cent; albumin/globulin, 2.2 per cent/6.8 per cent = 0.32; thrombocytes, 150,000; hemoglobin, 70 per cent; erythrocytes, 3.8 millions; color index, 0.83; leukocytes, 3,000. Differential count: segmented forms, 44 per cent; lymphocytes, 32.5 per cent; monocytes, 20 per cent; eosinophils, 2.5 per cent; basophils, 1 per cent. Urine: 1 plus albumin; Wassermann's test on blood, negative; blood pressure, 140/70 mm. Hg; blood urea, 67 mg. per cent increasing to 331 mg. per cent. Temperature up to 37.6° C. The patient died in uremia.

*Post-mortem examination* (no. 204/42) revealed, in addition to a right-sided lobar pneumonia and fibrinous pleuritis, alterations of the

kidneys with rough, flat depressions on the surface and irregular narrowing of the tissue border. The consistence was increased; the vessels were gaping. The right kidney was slightly larger than the left.

*Histologic examination* showed a chronic pyelonephritis and an extremely marked periarterial fibrosis in the spleen (Fig. 3), all follicular and penicillary arteries being surrounded by very broad, concentric, hyaline rings. In contrast with the usual findings in lupus erythematosus disseminatus, extraordinarily well defined plasma cell infiltrates (which stained according to Unna's method) were found around the hyaline rings and between the single lamellae of the latter. When Mallory's staining method was used, red lamellae were observed here and there between the others that had been stained blue. Jürgens' methyl violet stain and Congo red staining for amyloid each gave negative results.

A coincidence of hyperglobulinemia, periarterial fibrosis of the spleen, and marked plasmacytosis in the spleen was thus found in this case. There were no findings in support of a diagnosis of plasma cell myeloma or aleukemic plasma cell leukemia, in which periarterial fibrosis of the spleen is not seen either. Consequently, I considered this case to be one of allergic plasma cell reaction in the reticulo-endothelial system, with hyperglobulinosis and periarterial hyalinosis in the spleen, also with hyperglobulinemia.

The reactions in this case are similar to those of case 1 of *Letterer-Siwe's disease* reported in the preceding article (1948). The findings in that case were simultaneous hyperglobulinemia and hyalinosis (paramyloidosis). The concentric homogeneous rings surrounding the vessels (see Fig. 3) and marked accumulation of plasma cells are also similar.

In conformity with the common features of a number of conditions with stimulation of immune mechanisms, the periarterial fibrosis of the spleen observed in lupus erythematosus disseminatus can be considered morphogenetically parallel in all respects to the alterations found in Boeck's sarcoid, in so-called genuine amyloidosis (and paramyloidosis), and also in experimental amyloidosis after immunization; *i.e.*, it is a morphologic immunity reaction in the reticulo-endothelial system.

Examination of preparations from the case of *Boeck's sarcoid* in which post-mortem examination was made and which was reported in the preceding article (1948) revealed a marked periarterial fibrosis of the spleen (Fig. 4), in many places in close relation to the hyalin (paramyloid) developed around the epithelioid cell granulomata. In this case, too, numerous plasma cells and other reticulo-endothelial cells were observed between the lamellae. The hyaline deposits did

not stain like amyloid with Congo red, but with Mallory's staining method numerous fine and coarser red bands were found in the hyaline tissue that had been stained blue. Another striking feature is the resemblance between the concentric paramyloid rings in the peripheral parts of the granulomata in Boeck's sarcoid (Fig. 4 of the preceding article) in which numerous plasma and reticulum cells were embedded and the hyaline periarterial rings found in lupus erythematosus disseminatus.

### *Pathology of Lupus Erythematosus Disseminatus*

The periarterial hyalinosis referred to here takes a position of its own among the pathologic-anatomic alterations in lupus erythematosus disseminatus, partly owing to its constant localization, partly because of the sclerosing nature of the process. In these respects it is in contrast to the local changes observed in such cases in which there is evolution by stages with total, possibly fibrinoid, allergic necrosis, development of miliary granulomata, and secondary fibrosis (1945, 1946).

Another lesion of frequent occurrence and constant localization in lupus erythematosus disseminatus is the special alteration of the glomerular coils of the kidneys which has been previously characterized as "wire loops" because of the resemblance of the coils to bent wire (Baehr, Klemperer, and Schifrin). This is generally considered the most striking alteration of the kidney in this disorder. According to Klemperer, Pollack, and Baehr (1941, 1942), the coils are irregularly thickened and rigid, and are strongly eosinophilic. The thickening is found between the endothelium and the epithelium, apparently in relation to the basal membrane, resembling amyloid but failing to respond to all staining reactions for amyloid. In other cases, as in my case 1 (1945), there is *focal* fibrinoid necrosis of part of the glomerular coils, the others remaining unaffected. There seems to be no gradual transition between these two forms of alteration.

While the foci of necrosis of the glomerular coils are naturally considered analogous to the other scattered, allergic, in the narrowest sense, lesions, the "wire loop lesion" seems to be essentially different and its nature can probably be explained on the basis of the above-mentioned observations. For, if we grant that in such disorders as lupus erythematosus disseminatus, Boeck's sarcoid and atypical (genuine) amyloidosis there is, at any rate at certain stages, a hyalinosis of the reticuloendothelial system (especially of the spleen), in addition to a stimulation of immune mechanisms with hyperglobulinemia, it seems natural to accept the "wire loop lesion" as a hyalinosis analogous to the deposition of amyloid in the kidneys. Like Loeschcke, we may here reckon

with specific forms of hyalin, of which only the amyloid is open to a histologic characterization. In "paramyloidosis" the amyloid reactions are not constant either.

A relation between the wire loop lesion and the changes in so-called genuine amyloidosis may be illustrated by the following case.

### *Case 4. Polyarthritis Chronica with Atypical Amyloidosis*

The patient was a woman, 63 years of age, who had been admitted several times to Department A of the University Hospital of Copenhagen, service of Professor C. Sonne, for polyarthritis chronica rheumatica. She had never been affected with rheumatic fever, but had had angina tonsillaris several times a year and also recently. The onset of the patient's rheumatic disorder, in 1922, was associated with angina tonsillaris in the course of which she developed pain and swelling of the ankle joints, later spreading to other joints. About 18 months previously, she had had angina, fever, and albuminuria. Since then her condition had varied, but albuminuria had been present constantly. About 1 month before admission she had articular pain with increase of temperature to 39° C., in connection with influenza.

On examination a systolic murmur was found over the entire precordium in addition to swelling and tenderness of the joints. Blood pressure was 140/110 mm. Hg; hemoglobin, about 70 per cent; erythrocytes, 3.60 millions; leukocytes, 7,800; differential count, 61.5 per cent segmented forms, 4 per cent eosinophils, 1.5 per cent basophils, 25 per cent lymphocytes, 8 per cent monocytes. Wassermann test of the blood, negative; complement deviation reaction for gonococcal antibodies, negative.

On her first admission in 1942 the sedimentation test was from 34 to 91 mm. in 15 examinations made at regular intervals. On her second admission (from February 6 to May 1, 1946) it was found to be increasing evenly: Feb. 7, 82; Feb. 11, 91; Feb. 23, 92; March 2, 117; March 9, 107; March 13, 117; April 3, 144; April 13, 154; April 20, 140 mm. At the same time there was increasing *albuminuria* (up to 20 gm. per liter), increasing *blood urea* values (Feb. 7, 23; April 3, 82; April 13, 90 mg. per cent), and increasing *serum globulin* values:

| | Total protein *per cent* | Albumin *per cent* | Globulin *per cent* |
|---|---|---|---|
| 2/11/46 | 6.0 | 3.2 | 2.8 |
| 3/13/46 | 6.4 | 2.9 | 3.5 |
| 3/16/46 | 6.0 | 2.7 | 3.3 |

On her first admission, in 1942, the antistreptolysin titer was found to be increased (200); later it was normal.

*Post-mortem examination* (no. 212/46) showed pronounced brownish pigmentation of the skin, especially of the parts normally exposed to light. The *liver* was of normal size, with slightly increased consistence and giving a faintly positive amyloid reaction (iodine-potassium iodide). The *spleen* measured 15 by 8 by 4 cm., weighed 280 gm.; the cut surface was firm, elastic, and translucent. The *kidneys* were of normal size, displaying a slight granulation of the surface. The cortex had narrowed, and was pale. Spleen, kidneys, and suprarenal glands gave an intense amyloid reaction. Post-mortem diagnosis: Progressive polyarthritis; amyloidosis, marked in spleen, kidneys, and adrenals,

and mild in liver and small intestines; emaciation; melanoderma; bilateral hydrothorax; Addison's disease due to amyloidosis(?).

*Histologic examination.* In the periarterial zones to which the fibrosis is localized in lupus erythematosus, the *spleen* displayed homogeneous rings which assumed a purple-red color when stained according to Jürgens' method for amyloid. A similar substance was demonstrated in the intima, and in homogeneous clumps surrounding the vessels. There were numerous plasma cells.

The *kidneys* displayed marked glomerular alterations, with deposits of homogeneous substance between endothelium and epithelium in the coils, not so massive as they are normally seen in amyloidosis, but bearing a close resemblance to the wire loop lesion (Fig. 5). The substance assumed a blue color with Mallory's stain. With Jürgens' method it gave a faintly positive amyloid reaction. Congo red staining was negative. The amyloid and numerous casts in the tubules assumed a red color when stained according to Unna's method. The walls of the arterioles also contained large deposits.

In the course of a comparatively short time this patient presumably developed an atypical amyloidosis simultaneously with a highly increasing sedimentation reaction and an increase of the serum globulin. The spleen contained homogeneous deposits around the follicular arteries, in the kidneys deposits were found bearing a close resemblance to "the wire loop lesion" in lupus erythematosus disseminatus, and numerous homogeneous cylinders were present in the tubules staining in the same manner and presumably representing deposits of a globulin-like substance.

The underlying common immunity reaction was described in the preceding article (1948). Mention may also be made here of Stoeber's description (1934) of cases of allergic conditions combined with so-called genuine amyloidosis, and of Cazal's case of amyloidosis in a 7-year-old girl with "un état d'anaphylactique" as the only etiologic factor. It appears to me to be natural to associate both the splenic periarterial hyalinosis and "the wire loop lesion" in lupus erythematosus disseminatus with the allergic hyperglobulinosis of the reticuloendothelial system after the analogy of the conditions in atypical amyloidosis and paramyloidosis. In this way "the wire loop lesion" of the kidney would have to be considered a *glomerulonephrosis* like glomerular amyloidosis, consisting in the deposition of a globulin product between the endothelium and the epithelium in the capillary coils. Special immunobiologic conditions as well as the time factor must be supposed to be of importance in the development of the alterations and to their degree in the individual cases. It may be mentioned here that in the case of lupus erythematosus disseminatus previously de-

scribed by me (1945), and not displaying any signs of the wire loop lesion but widespread focal allergic lesions with necroses and development of granulomata, the symptom of hyperglobulinemia was lacking.

A distinction presumably should be made between different phases of the disease. A whole series of lesions which are allergic in the narrower sense (Teilum, 1946) are thus pathogenetically related to periarteritis nodosa, arteriolitis granulomatosa allergica, and to some extent resemble allergic reactions in serum sickness and experimental sulfathiazole intoxication described by Rich (1942) and in allergic syndromes of other nature (Bergstrand). In other cases the deposition of coagulable material resembling amyloid is the predominant feature, especially in the spleen and the glomeruli, but presumably also in the walls of the vessels. These last mentioned cases thus display morphogenetic features in common with "genuine" or atypical, and also experimental amyloidosis, and with the conditions with hyperglobulinemia, in particular Boeck's sarcoid, mentioned in the preceding publication. It is possible that we may in certain cases distinguish between an anergic form (positive anergy), in conformity with the conditions found in Boeck's sarcoid, and an allergic form in the narrower sense of the term, with preponderance of necrosis, possibly with cellular resorption and development of granulomata (Teilum, 1946).

Besides the relation already described between lupus erythematosus disseminatus and other allergic conditions (Teilum, 1946), a certain connection with such conditions as scleroderma, purpura haemorrhagica, and dermatomyositis (see Libman) also has been proposed. The connection between hyperglobulinemia, alterations in spleen and kidneys in lupus erythematosus disseminatus, and genuine and experimental amyloidosis seem to elucidate these conditions.

The occurrence of amyloid nodes in the skin has thus been demonstrated in clinical scleroderma (Lubarsch, case 1). In Goetz' case of generalized scleroderma there was thickening of the intima in the blood vessels with "fibrinoid" material and alterations resembling periarteritis nodosa, a "marked thickening and increase of the perivascular connective tissue" being demonstrated in the spleen. In a case clinically resembling Goetz' and described by Jörgensen, dermatomyositis-like alterations were found to be combined with widespread paramyloidosis and increase of plasma cells in the sternal marrow (20 per cent). The simultaneous occurrence of periarteritis nodosa and atypical amyloidosis has been described by Volland, and in "serum" horses with amyloidosis Doerken often found allergic intimal granulomata in the hepatic veins, resembling the granulomata of endophlebitis hepatica.

With regard to purpura hæmorrhagica, reference may be made to

the clinical picture of "purpura hyperglobulinemica" established by Waldenström, but purpura also has been described several times in atypical amyloidosis (*e.g.*, Lubarsch, case 2) in which many plasma cells were found in the spleen. Like hyperglobulinemia, purpura is also a symptom of frequent occurrence in lupus erythematosus disseminatus. Especially in cases of atypical amyloidosis with hyperglobulinemia the accumulation of plasma cells may be very considerable. Such a case which, like my case 3, might easily be considered one of plasma cell leukemia but for that accumulation, will be reported here.

*Case 5. Hyperglobulinotic (Paramyloid) Syndrome Caused by Sulfonamides*

A man, 58 years of age, was first admitted to Department A of the University Hospital of Copenhagen, service of Dr. C. Sonne, for examination because of albuminuria, ascertained 1 week before. He had no hematuria, hypertension, or immediately preceding infection. No previous renal disorder had been recognized. He had recently been suffering from fatigue and for the past 3 weeks from edema of the legs. Seven months previously he had had right-sided pneumonia with a protracted course in spite of treatment with sulfonamide (100 gm. in all). Pleuritis developed. When admitted for the second time he had constant albuminuria, about 12 gm. per liter. The blood showed a simple anemia. Hemoglobin had decreased from 96 to 55 per cent, and the sedimentation rate was increasing, from 50 to 100 mm. The formol-gel test was negative. There was no Bence-Jones protein in the urine. On Aug. 7, 1944, the total serum protein was 5.16 gm. per cent, with albumin, 2.36 gm. per cent, and globulin, 2.80 gm. per cent; on Dec. 19, 1945, serum protein was 7.5 gm. per cent, albumin, 3.10 gm. per cent, and *globulin, 4.4 gm. per cent.* Blood pressure varied from 90–125/50–90 mm. of Hg. Blood urea determinations were: Oct. 10, 1944, 40 mg. per cent; Dec. 11, 1945, 144 mg. per cent; Dec. 15, 1945, 156 mg. per cent. The patient died in uremia.

*Post-mortem examination* (no. 481/45) showed a widespread fibrinous pericarditis. The *heart* measured 11 by 11 cm., the weight being 440 gm. The wall of the left ventricle was 17 mm. thick. The myocardium was pale, without any macroscopic signs of fibrosis or myomalacia. Valves and ostia were normal. The *aorta* showed some atheromatosis. There was no ascites. The *stomach* displayed multiple fresh erosions. The *colon* showed no ulcerations. The *liver* measured 24 by 16 by 10 cm.; the surface was smooth and pale, and the cut surface had normal markings. The organ was of normal consistence. The *pancreas* and the *suprarenal glands* were normal. The *spleen* was enlarged, measuring 6 by 9 by 16 cm. Its cut surface was reddish and of a gritty consistence. The *kidneys* measured 3 by 5 by 11 cm., being slightly decreased in size. The surface displayed extensive, irregular, rather coarse depressions. The color was mottled deep red and pale red. On the cut surface the cortex was seen to be narrowed and somewhat spotted, the markings being blurred. The other organs were normal. The central nervous system was not examined.

*Histologic examination.* The glomerular coils of the *kidneys* displayed large homogeneous masses (Fig. 6) resembling amyloid, in some instances with secondary hyalinization. Similar precipitates were found everywhere in the walls of the small vessels as well as in those of the larger ones, forming thick homogeneous bands of a substance assuming a yellow-brown color when stained according to van Gieson's method. The tubules also contained deposits. Dense plasma cell infiltrates were scattered about and secondary fibrosis had occurred. The amyloid-like substance stained with Jürgens' amyloid stain and with Weigert's fibrin stain, but did not react with Congo red. With Unna's staining method it assumed a deep red color like that of the protoplasm of the plasma cells.

The *spleen* was the seat of diffuse plasma cell infiltration in the pulp. The walls of all vessels contained broad homogeneous bands (Fig. 7) like those of the renal vessels.

The *liver* contained accumulation of plasma cells, partly localized and partly diffusely scattered in the capillaries. The liver cells showed much fatty degeneration, and the vessels contained amyloid deposits which, like those of the other organs, caused a considerable thickening of the walls.

In the *myocardium* the same homogeneous deposits (Fig. 8) were found in the walls of the vessels, but plasma cells were few. There were incipient interstitial fibrosis and some interstitial leukocytic infiltration.

This was therefore a case of paramyloidosis with deposits in the glomeruli and in vessel walls of a number of organs. I have interpreted the alterations in this case as being expressive of a marked positive anergy (plasmacytosis with hyperglobulinosis and atypical amyloidosis). Morphogenetically the paramyloidosis must be considered analogous to the periarterial hyalinosis in case 3 in which there was also an allergic plasmacytosis.

The causal importance of sulfonamides to the paramyloid syndrome in this case (and to the development of allergic hyperglobulinosis in a case later on observed by me) forms a contrast to the well known hypersensitive reactions to sulfonamides. Together they represent reactions of two types which, as described above, form part of the pathology of lupus erythematosus disseminatus.

## Summary

Hyperglobulinemia, periarterial fibrosis of the spleen, and the wire loop lesion of the glomeruli in lupus erythematosus disseminatus are all considered to be expressive of a primary allergic hyperglobulinosis in the reticulo-endothelial system, after the analogy of the previously

described (1948) morphologic immunity reaction in atypical and experimental amyloidosis, Boeck's sarcoid, and other conditions.

Periarterial fibrosis of the spleen is thus produced in various conditions with hyperglobulinemia (with or without any demonstrable increase in the number of plasma cells) and displays all transitions to atypical amyloidosis, which, as an underlying cause, also has a stimulation of immune mechanisms with hyperglobulinemia.

Like the periarterial deposits and the collagenic sclerosis (diffuse scleroderma), the characteristic "wire loop lesion" occurring in many cases of lupus erythematosus disseminatus must be looked upon as alterations which are closely related pathogenetically to atypical amyloidosis; whereas focal, and in the narrowest sense allergic, lesions (miliary granulomata in the serosa, nodular necroses, cases of focal allergic pneumonia) are predominant in other cases, or may be present in addition to the lesions first mentioned. From the point of view of immunobiology these two groups of alterations may be considered expressive of a positive anergy and an allergy, respectively.

In some cases also, administration of sulfonamides may give rise to a hyperglobulinotic (paramyloid) syndrome (plasmacytosis, hyperglobulinosis, paramyloidosis in different organs, possibly uremia), in contrast to the well known hypersensitive reactions to sulfonamides.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

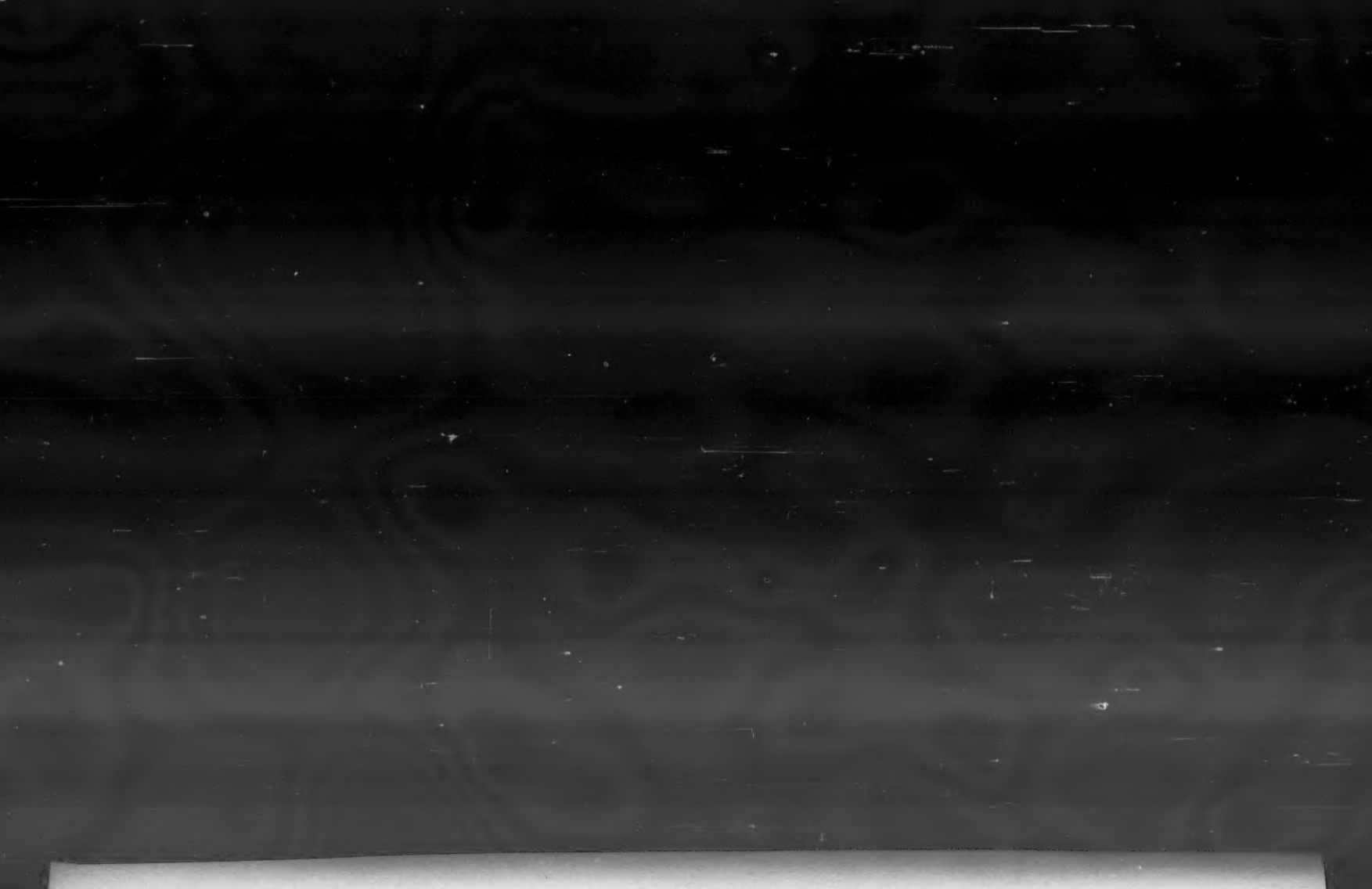
### PLATE 80

FIG. 1. Case 1. Periarterial fibrosis of the spleen in lupus erythematosus disseminatus. Concentric lamellar hyaline bands. van Gieson-Hansen's stain. × 220.

FIG. 2. Case 2. Periarterial fibrosis of the spleen in lupus erythematosus disseminatus. Between the rings a few reticulo-endothelial cells are seen. Hematoxylin and eosin stain. × 220.

FIG. 3. Case 3. Periarterial fibrosis of the spleen in allergic plasmacytosis of the reticulo-endothelial system with marked hyperglobulinemia. Marked accumulation of plasma cells in the border zone. Hematoxylin and eosin stain. × 220.

FIG. 4. Periarterial fibrosis of the spleen in Boeck's sarcoid with hyalinosis (paramyloidosis). Epithelioid cell granuloma with peripheral paramyloidosis is seen in the border zone. A number of plasma cells and other reticulo-endothelial cells. van Gieson-Hansen's stain. × 220.



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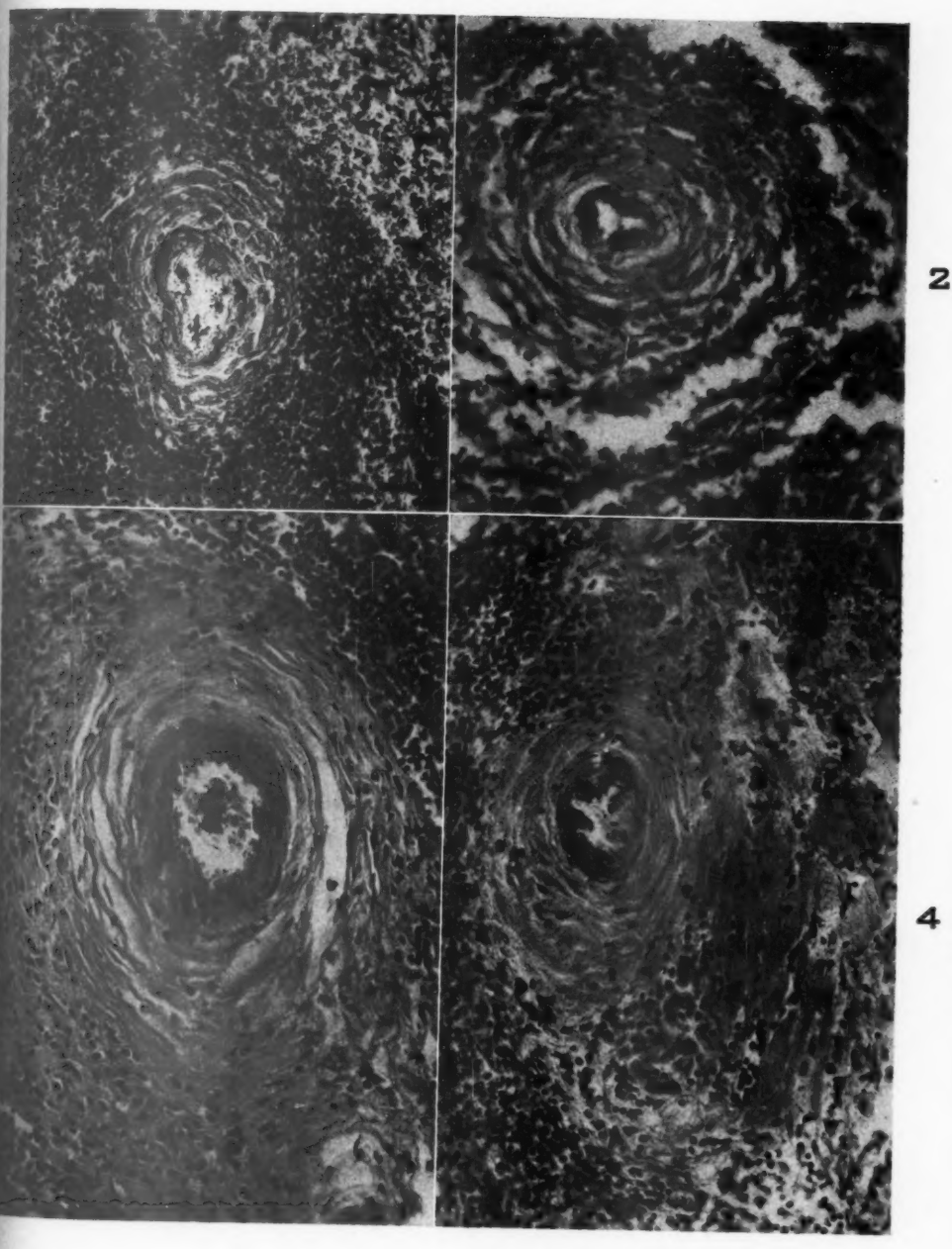


Teilum

Periarterial Fibrosis in Disseminated Lupus

PLATE 81

FIG. 5. Case 4. Glomerular lesions of chronic polyarthritis with atypical amyloidosis and hyperglobulinemia, for comparison with the wire loop lesion in lupus erythematosus disseminatus. Mallory's stain. × 270.

FIG. 6. Case 5. Glomerular lesions in allergic plasmacytosis with paramyloidosis and hyperglobulinemia, for comparison with the wire loop lesion in lupus erythematosus disseminatus. Hematoxylin and eosin stain. × 310.

FIG. 7. Case 5. Paramyloid deposits in the splenic vessels. van Gieson-Hansen's stain. × 220.

FIG. 8. Case 5. Paramyloid deposits in the vascular walls of the myocardium, Hematoxylin and eosin stain. × 120.

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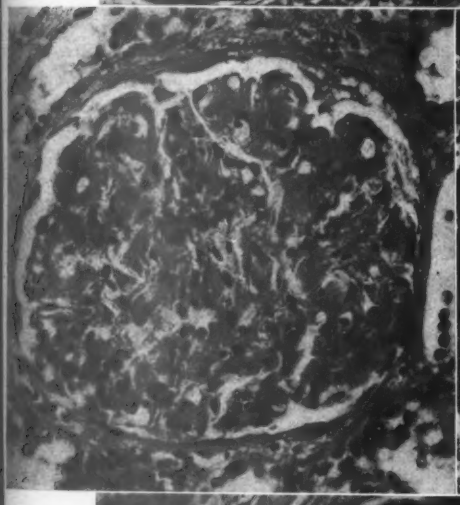

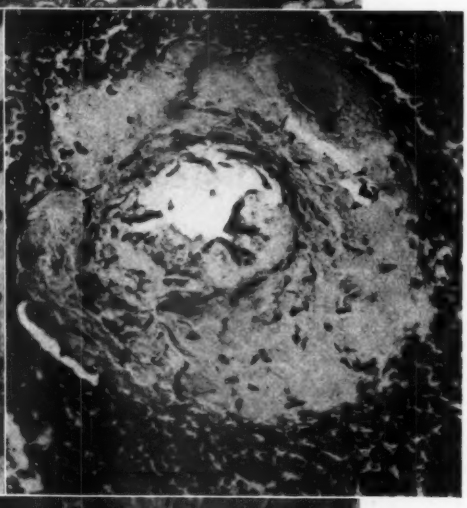

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Teilum

Periarterial Fibrosis in Disseminated Lupus

# EFFECTS OF ALLOXAN UPON FUNCTION AND STRUCTURE OF NORMAL AND NEOPLASTIC PANCREATIC ISLET CELLS IN MAN *

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Since the original demonstration in 1943 by Dunn, Sheehan, and McLetchie [1] that alloxan administered to rabbits produces selective necrosis of the pancreatic islets of Langerhans, it has been established that a single injection of this substance, varying from 50 to 400 mg. per kg. of body weight, is capable of producing destructive changes in the normal islets of the pigeon,[2] duck,[3] rat,[4,5] cat,[6] dog,[6] and monkey.[4,7] It has been found, too, that a series of smaller daily doses of alloxan produces islet cell degeneration but that the total dose required is considerably greater.[8,9] Under these circumstances the degenerative changes in the islet cells are less acute and evidence of attempted repair of damaged islets is observed in the rabbit.[8,10,11] Regeneration of damaged islets has been observed in the rabbit following a single small dose of alloxan.[12,13]

Published observations on the effects of alloxan upon the pancreas of man are limited to the 2 cases reported by Brunschwig *et al.*[14,15] In one case the pancreas was examined 7 hours after a single intravenous injection of 600 mg. per kg. of alloxan. In this case "microscopic study of the pancreas revealed questionable evidence of injury to a number of cells in some of the islets, although many islets were not affected." In the other, a case of islet cell carcinoma with metastases, no microscopic evidence of injury either of the normal islets or of the tumor cells was observed. This pancreas had been examined 1 month after the last injection of alloxan. In the preceding 50-day period the drug had been given in large doses (total 3,150 mg. per kg.) irregularly. Thus, in the 2 cases studied heretofore, no clear evidence of a destructive effect of alloxan upon human islet tissue has been observed. The impression has been gained, therefore, that the pancreatic islet cells of man are exceedingly resistant to the damaging effects of alloxan. That this conclusion is not warranted on the basis of so few observations is indicated by the fact that in our studies the sensitivity of human islet tissue to the destructive effects of alloxan approximates that observed for other species.[16] In addition, we found that neoplas-

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tic islet cells are much more resistant, both anatomically and functionally, to the damaging effects of alloxan than are normal islet cells.

Our observations were made upon a patient who satisfied all the criteria needed for a diagnosis of organic hyperinsulinism.[17] Subsequently, an islet cell tumor was shown to have been the cause of the hyperinsulinism. A brief summary of the history and clinical course of this patient follows.

## Report of Case

A.W., a white housewife, 58 years old, was admitted to the University Hospital on May 14, 1946, complaining of attacks of unconsciousness. Ten years previously she had noted the onset of severe early morning fatigue which was relieved by eating breakfast. This symptom gradually progressed in severity. Four years prior to admission pre-breakfast attacks appeared which were characterized by memory defects, blurring of vision, clouding of consciousness and, at times, inability to awaken in the morning. The great majority of such episodes occurred between 5 a.m. and 8 a.m. but occasionally one was experienced in the forenoon. The frequency and severity of the attacks continued to increase. During the year before admission attacks had occurred almost daily. In an effort to prevent attacks by eating frequently, the patient had gained 36 pounds during the preceding 3 years.

Except for obesity, the physical examination revealed no abnormalities. Blood pressure was 130/70 mm. of Hg.

The Kahn reaction of the blood was positive. Spinal fluid gave a negative Kahn reaction. Except for the abnormally low levels of the blood sugar outlined below, all other laboratory examinations showed no abnormality. These included blood counts, urine analyses, tests of kidney and liver function, x-rays of the skull and chest, and determinations of blood nonprotein nitrogen, basal metabolic rates, serum proteins, and plasma cholesterol.

## Procedures and Results

### *Summary of Metabolic Data*

From May 14, 1946, to November 25, 1946 (193 days), extensive metabolic data were obtained and will be reported in detail elsewhere.[18] For the purpose of integrating the functional and histologic changes observed, the major metabolic findings before, during, and after the administration of alloxan are outlined.

### Pre-alloxan Period (35 Days)

Daily fasting blood sugar ranged from 15 to 62 mg. per cent with an average level of 31 mg. per cent. Repeated oral and intravenous glucose tolerance tests, performed after standard dietary preparation,[19,20] demonstrated a greatly increased tolerance for carbohydrate. One meal low in carbohydrate (17 gm.) resulted shortly in intense hypoglycemia requiring glucose intravenously.

### Alloxan Period (9 Days)

Alloxan was administered intravenously on 9 consecutive days in the following amounts: 50, 50, 100, 100, 100, 100, 100, 100, and 100 mg.

per kg., for a total of 800 mg. per kg. The patient weighed 75 kg. and thus received 60 gm. of alloxan during the 9-day period. The diet was constant. The daily fasting blood sugar ranged from 29 to 54 mg. per cent with an average level of 40 mg. per cent. No untoward reactions occurred. Daily examination of the urine showed no albumin or cellular elements. For several hours after the administration of alloxan the urine was light red. No hemoglobin was present. The color was due to the excretion of a reduction product of alloxan. The blood nonprotein nitrogen and daily white blood cell count remained normal as did the bromsulfalein test for liver function.

### Post-alloxan Period

*Before Operation (15 Days).* The daily fasting blood sugar ranged from 34 to 60 mg. per cent with an average level of 45 mg. per cent. For the last 4 days of this period the levels were 41, 34, 45, and 35 mg. per cent, respectively. Repeated glucose tolerance tests done in this period indicated that despite the continuation of an abnormally low level of the fasting blood sugar, a marked decrease in carbohydrate tolerance had occurred as a result of alloxan administration. The persistence, however, of severe pre-breakfast hypoglycemic attacks made surgical exploration necessary.

*Operation.* On July 12, 1946, surgical removal of an islet cell tumor of the pancreas was performed by Dr. Robert W. Buxton, of the University Hospital staff. The tumor measured 1.5 by 1.0 by 1.0 cm., and was located in the body of the pancreas. Specimens of the pancreas proper and of the liver were removed for microscopic study.

*After Operation (134 Days).* Within 20 hours following removal of the islet cell tumor frank diabetes had developed with heavy glycosuria, ketonuria, and a fasting blood sugar of 208 mg. per cent. Insulin therapy was begun on the second postoperative day. Ketonuria disappeared in 2 days. Despite the use of 20 to 35 units of insulin daily (average, 30 units) and a low carbohydrate intake, the daily fasting blood sugar for the first 11 postoperative days ranged from 146 to 234 mg. per cent with an average level of 186 mg. per cent. An increase of insulin to as high as 60 units per day for several days brought the fasting blood sugar to normal where it remained for the next 16 days on a dose of 20 to 30 units per day. Insulin was gradually discontinued and the fasting blood sugar remained normal. However, postprandial glycosuria still occurred. Repeated glucose tolerance tests continued to show consistently normal fasting blood sugar levels, but they also demonstrated the hyperglycemic plateau type of curve typical of diabetes mellitus. From the character of serial curves it appears likely that normal tolerance for carbohydrate will eventually return.

### *Structural Changes*

*Methods of Preparation of Tissues.* The tumor was bisected, photographed and the halves were immediately placed in Bouin's fixative and in 10 per cent formalin. Small pieces of pancreas proper and of liver were placed in similar fixatives.

The following stains were used in the study of the tissues:

Hematoxylin and eosin stain
Mallory-Heidenhain azan stain
Mallory's phosphotungstic acid hematoxylin stain
Bensley's modification of Mallory's aniline blue stain
Ziehl-Neelsen's stain
Gomori's chromium hematoxylin-phloxine stain
Van Gieson's stain
Bensley's acid fuchsin and methyl green stain
Warthin-Starry-Kerr silver stain

*Evaluation of Staining Procedures Utilized.* The general histologic features were brought out nicely by the hematoxylin and eosin stain, Gomori's chromium hematoxylin-phloxine stain, Mallory's phosphotungstic acid hematoxylin stain, and the Mallory-Heidenhain azan stain.

The use of many sections of normal and diseased pancreatic tissue as controls proved that Gomori's [21,22] stain distinguished beta cells from those of the alpha and delta types more efficiently than other stains utilized. By this technic the normal beta cells possessed a very fine, dust-like, blue granular cytoplasm whereas the alpha and delta cells were indistinguishable from each other and had a pink to red, more coarsely granular cytoplasm.

The identity of alpha cells was confirmed by Mallory's phosphotungstic acid hematoxylin stain.[22] The alpha granules took a dark blue stain. These cells corresponded to the alpha cells by the Gomori stain. The Mallory technic (phosphotungstic acid hematoxylin) differentiated alpha from delta cells since the latter were not stained by this method.[22]

The Mallory-Heidenhain azan stain was useful but alpha and delta cells failed to stain as brilliantly as reported by Gomori.[22] Beta cells stained a pale gray, while delta cells were blue.

Bensley's procedures were not done on fresh material, but those recommended for fixed material [23,24] were uniformly unsatisfactory in our hands.

The Warthin-Starry-Kerr method[25] and Ziehl-Neelsen's stains were negative for organisms. Van Gieson's stain was used for study of the hyaline material, which took a pale yellow to pale pink color.

Gomori's chromium hematoxylin-phloxine stain was used with slight modifications in timing. The hematoxylin solution was allowed to age for at least 1 week instead of 48 hours as recommended. Sections were exposed to both staining solutions about twice as long as Gomori [22] suggested and differentiation and decolorization procedures were corre-

spondingly increased. Greater contrast and better detail were obtained by this longer procedure.

### Islet Cell Counts

The islets of the pancreas proper were segregated according to the degree of damage which they exhibited and the component cells were studied and tabulated according to qualitative and quantitative changes. Inasmuch as the cells of the adenoma failed to show the qualitative and quantitative changes characteristic of alloxan injury, these cells were not counted individually but an approximation was made of the frequency of cell types on the basis of the granule stains.

### Gross Features of the Islet Cell Neoplasm

*In situ* the tumor presented as a slight protrusion above the body of the pancreas. The neoplasm was slightly firmer than the surrounding pancreas. A rich vascular network coursed over its surface, a feature which aided in distinguishing the tumor from enlarged peripancreatic lymph nodes.

Upon removal and sectioning, the tumor was found to be coarsely lobulated. It weighed about 1.5 gm., a weight which is equivalent to the estimated combined weight of all of the islets in the average normal pancreas.[26] It measured 1.5 by 1.0 by 1.0 cm. Most of the periphery was well encapsulated and well demarcated from adjacent pancreatic tissue. However, localized peripheral areas showed no distinct capsule. The cut surface was primarily a dull gray with irregular blotches of dull reddish purple.

### Histopathologic Features of the Islet Cell Tumor

With regard to general architecture and cellular type, the tumor removed from this patient conformed in all respects to the numerous excellent descriptions which are readily available in the literature.[27-33] Suffice it to say that the pattern of cells and their structure were almost identical with those observed in normal or in hyperplastic islets of Langerhans. The following description is aimed at emphasizing (1) the possible genesis of the neoplasm, (2) age differences in various portions of the tumor, (3) cell types according to specific granule stains, and (4) the complete absence within the tumor of evidence of alloxan injury.

In the areas where the fibrous connective tissue capsule was lacking, multiple small nodules representing centers of active proliferation were found. Many of these proliferating nodules had surrounded and compressed acinar tissue, islets, and fat lobules. Many ducts and ductules were seen in intimate relation with the tumor cells. Ductules possess-

ing terminal bars and relatively heavy basement membranes were easily identified in these nodules. Commonly these ductules were seen to end in the tumor. The epithelium of the ductules blended with and appeared to give rise to the cells of the neoplasm. At least 70 per cent of the cells in some of these small nodules appeared to be the epithelial cells of ductules. They were indistinguishable from such epithelial cells in size, shape, cytoplasmic granules, and staining reactions. These cells were larger than normal islet cells but were somewhat smaller than most of the cells of the neoplasm. They also possessed a darker staining cytoplasm than most of the cells of the tumor. They were columnar or polyhedral. Cytoplasmic granules were coarser than those of beta cells. These granules showed no particular affinity for either of the specific colors in the granule stains. Scattered among these undifferentiated cells were a significant number of cells showing transformation to a beta-like cell in that a varying degree of development of granules of the beta type were seen within them. Mitotic figures could not be found in these or other portions of the tumor. On the basis of these findings, it was considered that the neoplastic islet cells seen in this tumor had their origin in duct epithelium.

The larger and older nodules of the tumor showed few ducts and little evidence of proliferation or of transformation of duct epithelium to tumor cells. Dense hyaline tissue was considerably more abundant in these areas.

Eighty-five to 90 per cent of the cells resembled beta cells in their staining reactions. Approximately 10 per cent of these possessed their full complement of granules. The remaining cells of the beta type demonstrated varying degrees of degranulation. Less than 1 per cent of the cells contained granules stainable like those of alpha cells, either by Gomori's method or by Mallory's phosphotungstic acid hematoxylin stain. The rest of the cells were either degenerating tumor cells showing hyalinization or they were undifferentiated cells which could not be classified. Surrounding the tumor and in the interstices of the pancreas were foci of heavy inflammatory reaction.

The most striking feature of the cells of this tumor was the complete absence of destructive changes which could be attributed to alloxan injury. In marked contrast were the intense degenerative changes, characteristic of alloxan injury, which were observed in the islets of the pancreas proper.

### Histopathologic Features of the Pancreatic Islets

Virtually every islet in the pancreas showed some degree of damage attributable to alloxan injury. The degree of change varied greatly

from islet to islet. When examined after staining with routine hematoxylin and eosin the extent of the damage seemed slight. Islets were small and some of the cells showed vacuolar degeneration. However, the specific granule stains revealed profound changes both qualitatively and quantitatively.

The qualitative changes, which predominantly involved the beta cells, were many and varied. Minor degrees of alteration (some of which were probably not the result of alloxan *per se*) included degranulation, marked cloudy swelling, vacuolar degeneration, and other cytoplasmic disruptions. More severe changes involved loss of cytoplasm and cell membranes, pyknosis and fragmentation of nuclei proceeding to necrosis and disappearance of cells. With disruption of cell membranes and cytoplasmic boundaries there was collapse of entire islets. If severe, this process resulted in small shrunken masses of cells without the usual complexity of architecture seen in normal islets. Entire islets were destroyed leaving only a few shadows of former islet cells, or a few disorganized cells among cellular débris. However, the amount of cellular débris was small in comparison with the severe destruction observed, indicating rapid removal of the destroyed cells.

Normally the average island of Langerhans contains at least as many or more beta cells than alpha and delta cells combined [21] and evidence of degeneration is lacking. The changes which we observed in these islets included the complete disruption of the normal ratio of alpha to beta cells. Careful cell counts of 128 islets showed that 82 per cent were alpha or delta cells. Only 18 per cent were beta cells. Of these, 89 per cent showed significant degenerative changes. Thus, less than 2 per cent of all of the cells studied in the islets could be considered to be normal beta cells. With a normal expectancy of at least 50 per cent normal beta cells, this represented a degeneration and/or disappearance of 96 per cent of all of the beta cells. For purposes of classification and of comparative cell counting, the islets were divided into five groups:

1. "Least injured" (approximately normal size and architecture).
2. "Collapsed" (moderate loss of cells and disruption of normal pattern).
3. "Shrunken" (severe loss of cells and disruption of normal pattern).
4. "Completely destroyed" (remnants and débris indicating the site of a former islet).
5. "Hyperplastic" (enlarged, increased number of cells and exaggerated ribbon pattern).

1. As will be noted in Table I, 42 islets (33 per cent) were "least

injured." In only 4 of these did the ratio of alpha + delta to beta cells fall within the normal range. In these 4 islets 53 per cent of cells were beta cells. But 52 per cent of these beta cells demonstrated significant destructive changes, such as marked cloudy swelling, vacuolar change, pyknosis, and necrosis. Forty-seven per cent of the cells belonged to the alpha-delta series and appeared to be unharmed.

TABLE I

*Qualitative and Quantitative Analysis of All Types of Islet Cells Encountered in 128 Islets of Langerhans (15 Days after the Administration of Alloxan)*

| Classification of 128 islets studied | Number of islets in each group | Disappearance of beta cells, average percentile decrease per islet* | Condition of remaining beta cells | | Condition of alpha and delta cells |
|---|---|---|---|---|---|
| | | | Normal | Degenerative changes | |
| Least injured | 42 (33%) | 4 islets 0% | 48% | 52% | Normal |
| | | 38 islets 22% | 23% | 77% | Mild degenerative changes |
| Collapsed | 27 (21%) | 72% | 7% | 93% | Mild degenerative changes |
| Shrunken | 33 (26%) | 88% | 0 | 100% | Moderate degenerative changes |
| Completely destroyed (remnants and débris) | 19 (15%) | 100% | | | Disappearance of recognizable cells |
| Hyperplastic | 7 (5%) | 80% | 26% | 74% | Marked hyperplasia with brilliant staining (newly formed cells) |

* Based upon the maximal normal ratio, $\frac{\text{alpha + delta cells}}{\text{beta cells}} = 1.0$.

The other 38 islets in this "least injured" group revealed a decrease in beta cells with a greater number showing destructive changes. In this group there were 39 per cent beta cells of which 77 per cent showed destructive changes. On the basis of the least expected number of beta cells in an unchanged islet there had been complete destruction and disappearance of at least 22 per cent of the beta cells. Sixty-one per cent of the cells were of the alpha-delta series. The granules of these alpha cells revealed a somewhat reduced affinity for the specific stains and occasionally they showed moderate cloudy swelling.

2. Islets showing varying degrees of "collapse" composed 21 per cent of the total islets counted. Only 14 per cent of the cells were beta cells, a figure which is much reduced from the 39 per cent seen in the previous group of islets. At least 72 per cent of the beta cells had been

completely destroyed. Of the relatively few beta cells remaining, a greater number (93 per cent) demonstrated significant degenerative changes. Many cells of the alpha-delta series in this group of islets also showed mild degenerative changes.

3. Twenty-six per cent of the total number of islets showed severe destructive changes resulting in "shrunken" islets. Of the cells present, only 6 per cent were beta cells, representing a complete disappearance of at least 88 per cent of beta cells. Ninety-three per cent of the cells of the islets were alpha and delta cells. The staining of the cytoplasmic granules in these alpha-delta cells was definitely impaired as compared with normal alpha and delta cells. In the severely shrunken islets many of the pink-staining cells were small spindle-shaped structures with little cytoplasm.

4. Fifteen per cent of the islets were completely destroyed. Perhaps many more belonged in this category, but sufficient proof of former islet architecture was lacking. Only a few disrupted islet cells or their "ghosts" were present. Sometimes a few spindle-shaped pink-staining cells and numerous ductules were observed. Nearly all of the cells seen stained pink and contained no definite granules.

5. Five per cent of the islets demonstrated the typical exaggerated ribbon pattern of hyperplastic islets. Strikingly, about 90 per cent of the cells in these islets were alpha and delta cells; and, of these, an unusual number were delta cells as shown by Mallory's phosphotungstic acid hematoxylin method. By this method and by other specific granule stains, the alpha and delta granules of the cells of these hyperplastic islets took a very brilliant stain suggesting that these cells were not present during the destructive insult which decreased the stainability of the older alpha and delta cells, and that this may represent regenerative proliferation of alpha and delta cells. The presence of an increased number of ducts within and surrounding the hyperplastic islets supports this opinion. Seventy-four per cent of the few beta cells which were present (10 per cent of the total in the hyperplastic islets) revealed degenerative changes.

### *Liver Obtained for Biopsy*

The liver cells were well filled with glycogen. They showed no evidence of injury. The liver lobules were intact and appeared to be normal. There was, however, an increase in young connective tissue in the portal trinities with an accompanying slight inflammatory infiltration of lymphocytes and mononuclear cells. Several tubercle-like foci of epithelioid cells were encountered. They bore a very close resemblance to those observed by Dunn and co-workers [34] in the

regional lymphoid tissue of a rabbit after the intravenous administration of alloxan.

## Discussion

There can be no doubt that the severe degenerative changes that we observed in the pancreatic islets of this patient were the direct result of the alloxan which had been administered. The changes are typical of those observed in the islets of alloxan-treated animals. They resemble most closely those described by Bailey *et al.*[8] as occurring in rabbits given small daily injections of alloxan for from 7 to 13 days.

The intensity of the alloxan-induced damage to the beta cells of the islets is indicated by the facts that (1) 64 per cent of all the beta cells had disappeared, (2) of the beta cells remaining, 90 per cent showed degenerative changes, and (3) only 2 per cent of all of the cells seen in the islets could be regarded histologically as normal beta cells. This degree of structural damage to the pancreatic islets of a human being resulted from the administration of a total of 60 gm. of alloxan (800 mg. per kg. of body weight) given intravenously in divided doses over a 9-day period. The amount of alloxan (on a per kg. basis) required to produce these changes falls close to that needed to produce similar islet cell damage in other species when the drug is given in daily divided doses.[8,9] One is forced to conclude, therefore, that in this study the sensitivity of human pancreatic islet tissue to the destructive effects of alloxan approximates that observed for other species. This is in sharp contrast to the statement found repeatedly in the literature to the effect that the pancreatic islet tissue of man is resistant to the damaging effects of alloxan. This conclusion is based upon the observations of Brunschwig *et al.*[14,15] in 2 cases in which the pancreas was examined after the administration of alloxan. While there is no ready explanation for the discrepancy between our findings and theirs, one may suggest several possibilities.

It is recognized that an occasional animal of any species is found to be resistant to the destructive effects of alloxan.[6,35,36] It is conceivable that Brunschwig's first patient,[15] who had received tremendous amounts of alloxan, fell into such a category. The other patient,[15] having received a single dose of alloxan (600 mg. per kg. intravenously), died 7 hours later. Questionable histologic evidence of injury was reported. It is possible that more positive evidence of islet cell damage would have been observed had the patient survived for a longer period of time. In this connection, it is of interest that we, too, have observed early degenerative changes in the pancreatic islets of a patient who died 9 hours after a single intravenous dose of alloxan amounting to 215 mg. per kg.[37] This patient had been suffering from

long-standing organic hyperinsulinism, shown at autopsy to have been due to marked generalized hyperplasia of the islets of Langerhans. He was moribund upon admission to the hospital and death could not be attributed to the alloxan which had been administered.

Thus it appears premature to conclude that human pancreatic islet tissue is less susceptible to damage from alloxan than is islet tissue of every other species studied. This point is of considerable importance with regard to present and future investigations upon the possible rôle of endogenous alloxan or related compounds in the etiology of some types of diabetes mellitus in man. The "resistance" to alloxan of human islet cells has been a potent argument against such speculations.

Of both practical and theoretical interest is the fact that human *neoplastic* islet tissue is very much more resistant to the destructive effects of alloxan than is normal islet tissue. In this study the normal islet cells have served as a control for the neoplastic ones. Both were exposed to the same insult. While very few normal beta cells remained in the islets of Langerhans, the beta-like cells of the tumor showed no evidence of alloxan injury. Since it has been reasonably well established that alloxan exerts its injurious effect upon cellular metabolism, by virtue of its capacity to oxidize sulfhydryl groups, thus interfering with certain essential intracellular enzymatic oxidation-reduction systems,[38] it would appear that the metabolism of neoplastic islet tissue differs in important respects from that of normal islet cells. It is suggested, further, that the hypoglycemia-invoking product secreted by neoplastic islet cells may not be identical with the crystalline protein substance obtainable from normal islet tissue.

From the practical point of view it would appear that attempts to degenerate islet cell neoplasms by means of the administration of alloxan are fraught with danger. It must be realized first that were the attempt successful it almost certainly would be associated with complete degeneration of all of the normal insulin-producing islet tissue in the body. It is more than likely, however, that the amount of alloxan required (if it affected neoplastic islet tissue at all) would be lethal to the patient, since the compound is injurious to tissues generally.

The metabolic studies made upon this patient will be reported in detail elsewhere.[18] They confirm, from a functional point of view, all of the conclusions which can be drawn from study of the structural alterations described. Following the administration of alloxan there occurred a remarkable decrease in the patient's ability to metabolize ingested carbohydrate. The degree of impaired carbohydrate tolerance was comparable to that seen in diabetes mellitus. The striking point

of difference in this patient, however, was the persistence of very low levels of daily fasting blood sugar (pre-breakfast level). On the basis of these findings it was concluded clinically that the islet cell tumor (which is responsible for the pre-breakfast hypoglycemia always observed in these patients)[17] had been but little, or not at all, affected by alloxan. It was reasoned that the sharp decrease which had occurred in the patient's tolerance for carbohydrate must have been due to injury of normal islet tissue. These conclusions seemed warranted in the presence of continued normal hepatic function. The prompt development of typical and persistent diabetes mellitus following surgical removal of the islet cell tumor completed the clinical story and related it in all of its details to the anatomic observations described.

Several other observations from the structural point of view seem worthy of comment. An occasional islet cell in the pancreas proper was observed to be undergoing hydropic degeneration. This is the type of islet cell degeneration which has been associated experimentally with procedures known to place excessive functional demands upon the islets,[39,40] a form of "overwork degeneration." This change is not usually found in animals as the result of acute alloxan injury, although it has occasionally been observed.[8] It seems likely that this change when it occurs is not a direct but an indirect effect of alloxan. Those beta cells which have escaped the damaging effects of alloxan are now subject to excessive functional strain and may undergo "overwork degeneration."

Although the functions of the alpha and delta cells of the islets of Langerhans are not known, two types of change involving these cells were observed in our sections. It was noted (Table I) that in those islets that suffered the greatest degree of beta cell injury, the alpha and delta cells showed mild to moderate decrease in stain-affinity, suggesting a slight degree of injury. The other change was related to the presence of occasional hyperplastic islets. Ninety per cent of the cells of these islets were alpha and delta cells. The unusually brilliant staining of these cells and the increased numbers of ducts surrounding these hyperplastic islets suggest that the large islets represent a proliferative response of alpha and delta cells which followed the alloxan injury.

Finally, sections of the beta-cell-like tumor indicate strongly that it arose from duct epithelium. The question of the origin of islet cells from duct epithelium has been a controversial one since Bensley [23] and Grauer [41] first suggested it. Direct continuity between duct epithelium and cells of islet tumors has been observed repeatedly.[28,29,42-44] These observations have led Laidlow [28] and O'Leary and Womack [29] to postulate that islet cell tumors originate in response to a stimulus to duct

epithelium, and that this particular stimulus calls forth the islet cell potentiality of duct epithelium. There is much to support the opinion that the neoplasm in our case, secreting an insulin-like substance, arose from and continued to receive from duct epithelium, undifferentiated cells which matured to become beta-cell-like tumor cells.

## Conclusions

1. In this study the sensitivity of normal human islet tissue to the destructive effects of alloxan was found to approximate that observed in other species.

2. Neoplastic islet tissue is much more resistant to the injurious effects of alloxan than is normal islet tissue. This suggests that certain metabolic processes within the abnormal cells differ significantly from those which exist in normal islet cells.

3. The development of persistent diabetes mellitus following surgical removal of the uninjured islet cell neoplasm confirms functionally (a) the severe structural lesion observed in the islets of the pancreas proper and (b) the absence of structural alterations in the tumor cells.

4. In future attempts to use alloxan therapeutically the above facts should be considered. It is believed that the amount of alloxan required to produce the desired effect upon islet cell neoplasms is likely to be lethal to the patient.

5. Alpha and delta cells of human islet tissue are susceptible to damage by alloxan, but to a much lesser degree than are beta cells.

6. Hyperplasia of alpha and delta cells appears to be one response to alloxan injury of the islets of Langerhans.

7. In this case the evidence is good that the islet cell tumor had its origin in pancreatic duct epithelium.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

### PLATE 82

FIG. 1. Relatively normal or "least injured" islet of Langerhans showing normal size, structure, and ratio of alpha+delta to beta cells. The cells with dark cytoplasm are normal beta cells. Scattered beta cells show significant degenerative changes and actual necrosis. Gomori's chromium hematoxylin-phloxine stain. × 125.

FIG. 2. A moderately collapsed islet with smaller size and somewhat altered structure composed largely of alpha and delta cells. Small masses of cellular débris represent destroyed beta cells. A large number of remaining beta cells show severe degenerative changes as described. Gomori's chromium hematoxylin-phloxine stain. × 125.

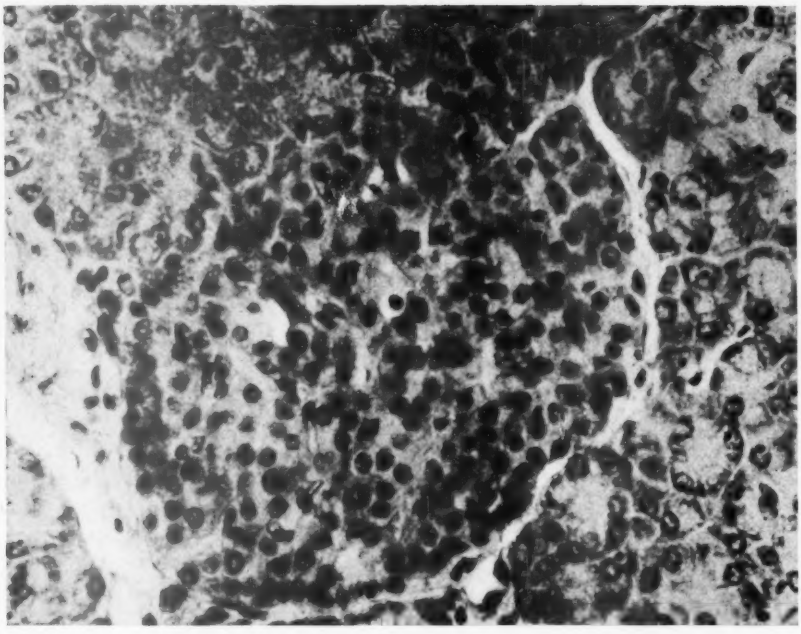
A

1

2

C

1



2

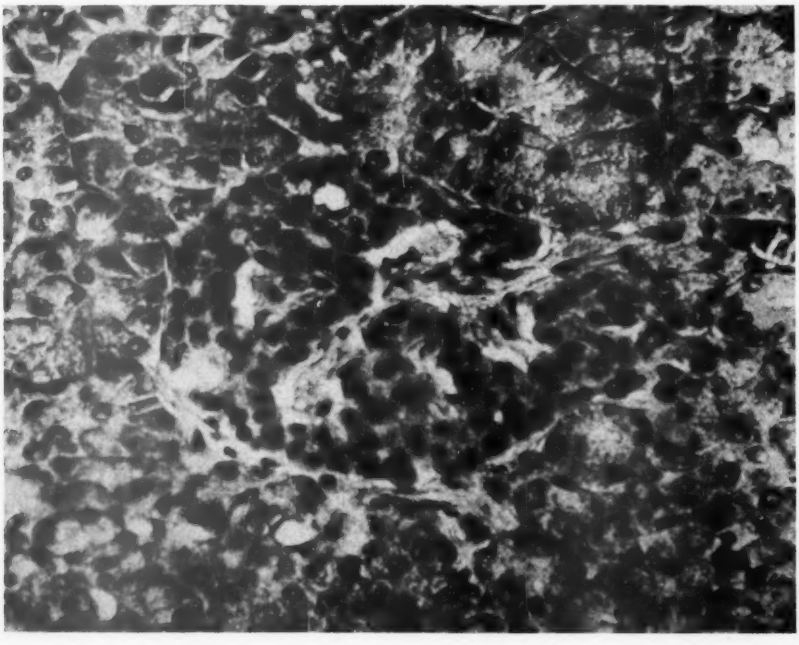


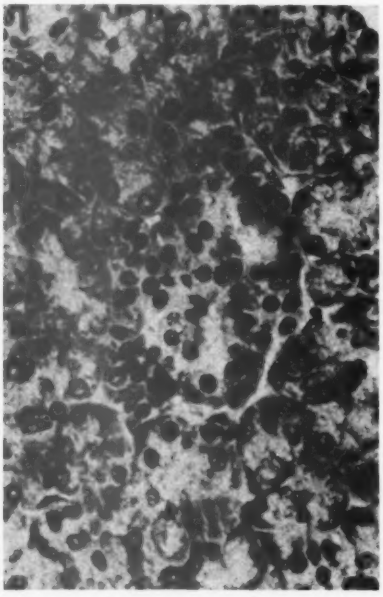
PLATE 83

FIG. 3. Small islet showing a more severe degree of collapse. The centrally arranged beta cells are largely necrotic. The peripherally arranged alpha and delta cells show slight degenerative changes and impaired stainability of granules. There is shrinkage of the entire islet structure. Gomori's chromium hematoxylin-phloxine stain. × 125.

FIG. 4. A greatly shrunken islet composed largely of a reduced number of spindle cells possessing a pale pink cytoplasm. No normal beta cells are found. Gomori's chromium hematoxylin-phloxine stain. × 125.

FIG. 5. A completely destroyed islet showing only connective tissue hyalin outlining the former islet pattern, and a few isolated bizarre cells. Gomori's chromium hematoxylin-phloxine stain. × 125.

3



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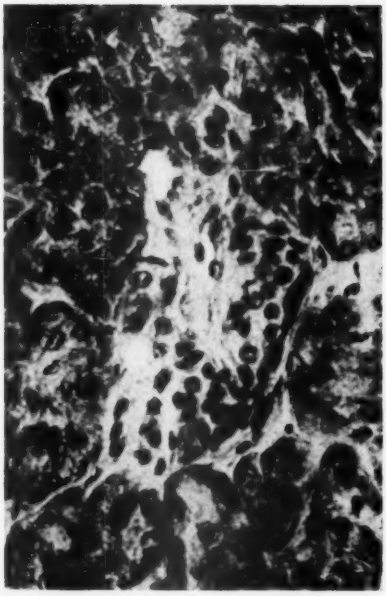

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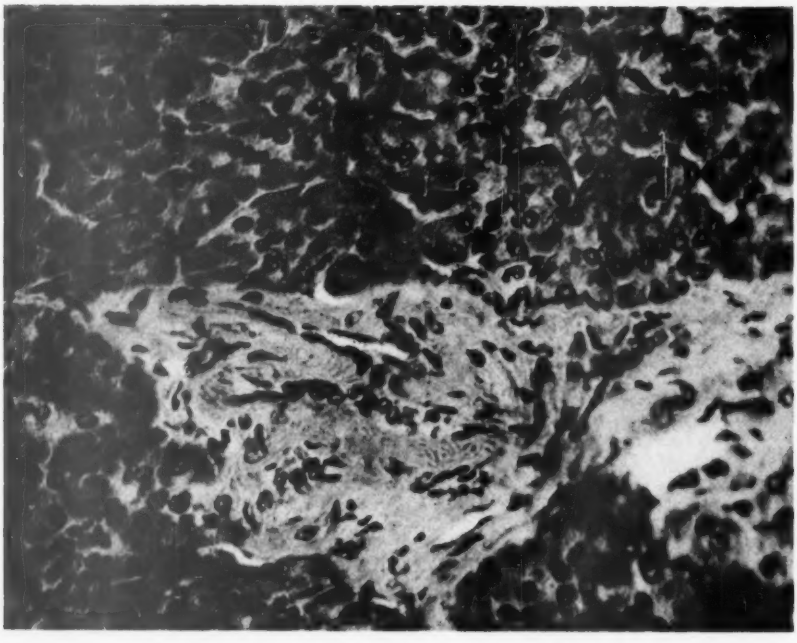
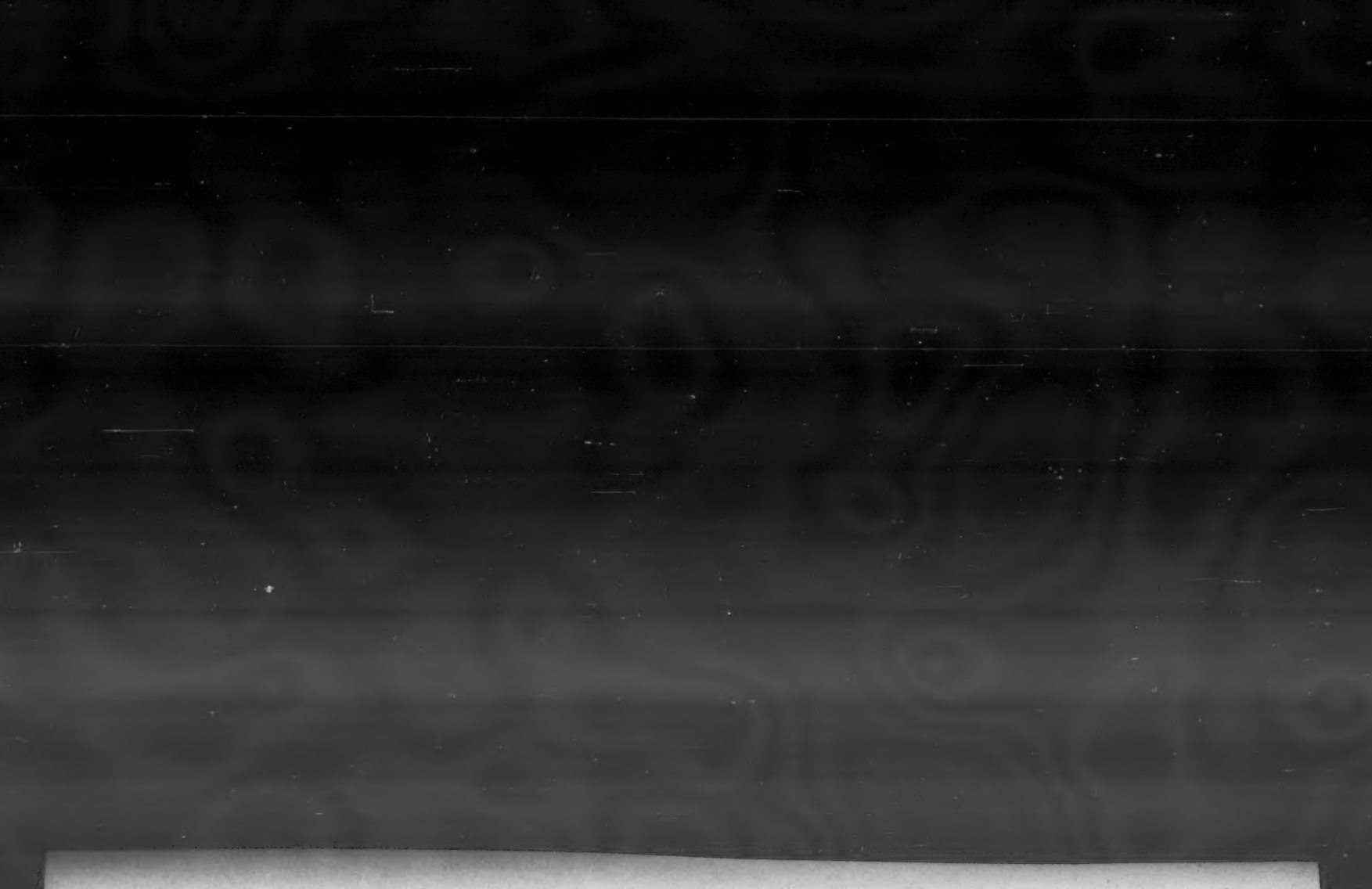


PLATE 84

FIG. 6. Photomicrograph showing young peripheral tumor cells, (A); duct epithelium, (B); relatively undamaged islet of Langerhans, (C); and acinar epithelium, (D). The young neoplastic cells resemble duct epithelium. Faint cytoplasmic stippling of tumor cells represents beginning beta-like granulation. The tumor cells appear in close relation to duct structures. Gomori's chromium hematoxylin-phloxine stain. × 125.

FIG. 7. Tubercle-like epithelioid focus in the liver apparently due to alloxan. Mallory-Heidenhain azan stain. × 125.



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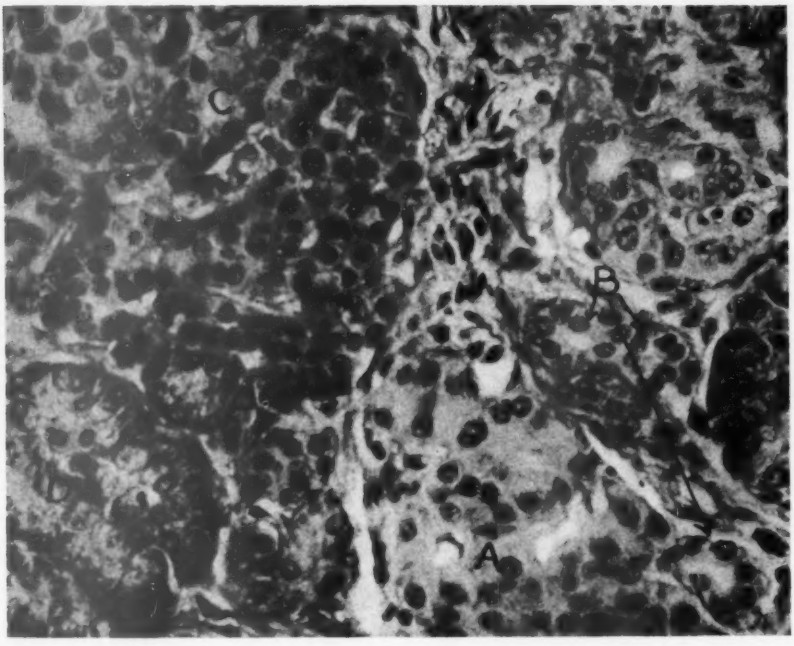

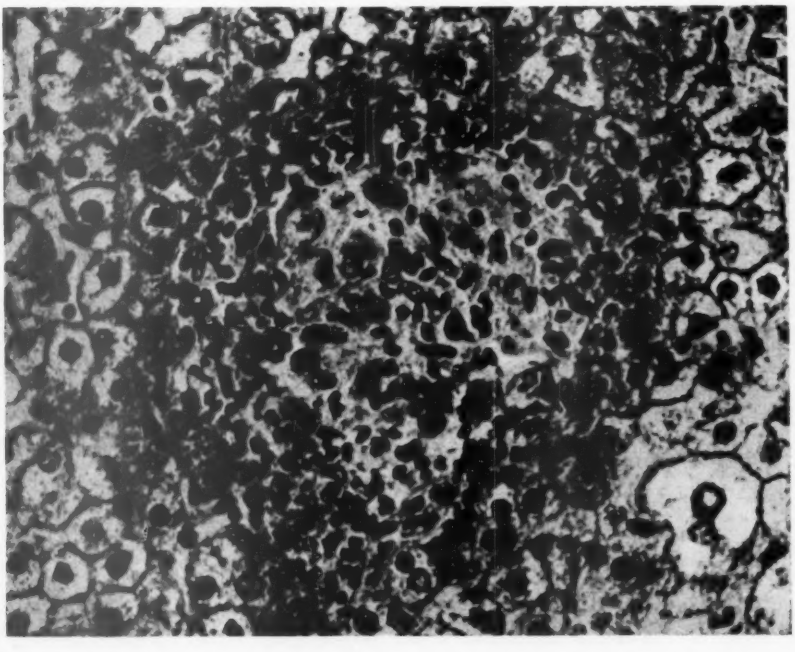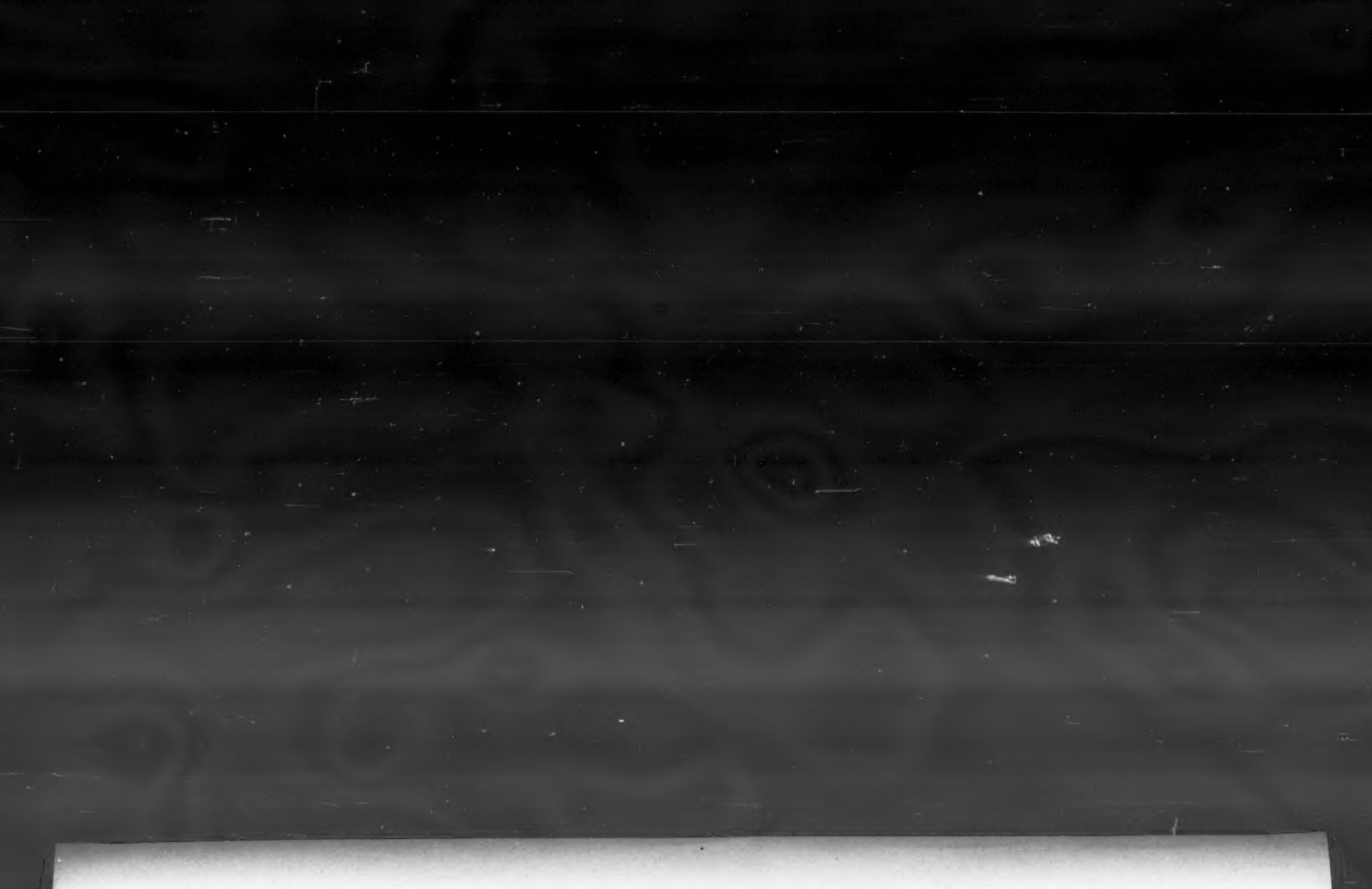
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